

**DIRECT ASSESSMENT OF THE FIXATION AND RELEASE OF NITROGEN BY  
PEA, LENTIL, CHICKPEA AND FABA BEAN**

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For the Degree of Master of Science

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University of Saskatchewan

Saskatoon

By

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## ABSTRACT

Pulse crops make significant contributions to the economy and environmental sustainability of western Canadian cropping systems. Due to their ability to provide N and non-N benefits to subsequently grown crops, pulse crops play a key role in crop rotations. The aim of this study was to trace the fate of fixed N in a pulse–cereal crop sequence. The pulse phase of the study involved quantifying the amounts of fixed nitrogen in the above- and below-ground plant compartments of chickpea, faba bean, lentil and pea; and evaluate their contribution to soil nitrogen pools. Plants were labelled with  $^{15}\text{N}_2$  using a custom-built soil atmosphere labelling system. The amount of biologically fixed N in the above-ground biomass of the pulse crops was greatest for faba bean and chickpea (82%Ndfa), intermediate for lentil (70%Ndfa), and least for pea (28%Ndfa). Conversely, the percentage of fixed N in the below-ground biomass (i.e., roots) was greatest for the chickpea (58%Ndfa), intermediate for the faba bean (37%Ndfa) and lentil (32%Ndfa), and least for the pea (18%Ndfa). Total amounts of fixed N returned to the soil as crop residue increased in the order: pea (12 mg N pot<sup>-1</sup>) << lentil (193 mg N pot<sup>-1</sup>) << faba bean (375 mg N pot<sup>-1</sup>) < chickpea (556 mg N pot<sup>-1</sup>). The cereal phase of the study was carried out in a greenhouse to quantify the nitrogen contribution of the pulse residues to (i) the subsequent wheat crop, and (ii) nitrous oxide (N<sub>2</sub>O) emissions from the soils. The residue-amended soils were first frozen (-20°C) and then slowly thawed at room temperature. Gas sampling was initiated during the thaw period, continued for 10 weeks, and was terminated two weeks prior to harvest—with the gas samples analyzed for total and  $^{15}\text{N}_2\text{O}$  concentration. Wheat yields were about 63% greater when the previous crop was a pulse compared to when it was wheat. Recovery of fixed-N (i.e., residue-derived N) by the wheat ranged from 9% to 14% for the pulse crops, compared to recovery of only about 4% residue-derived N when the previous crop was wheat. Soils amended with pulse residue, but not wheat residue, exhibited significant N<sub>2</sub>O emissions (total and  $^{15}\text{N}_2\text{O}$ ) during the thaw period, which is indicative of greater (faster) turnover of the pulse residues. Residue-derived emissions also showed a strong positive correlation ( $r = 0.954$ ;  $P = 0.012$ ) with the amount of residue-N added to the soil.

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## LIST OF ABBREVIATIONS

%Ndfa	Percentage nitrogen derived from atmosphere
%NdfR	Percentage nitrogen derived from rhizodeposition
%NdfR	Percentage nitrogen derived from residue
AFPS	Air-filled pore space
<i>amoA</i>	Gene encoding for the production of ammonia monooxygenase
AOA	Ammonia oxidizing archaea
AOB	Ammonia-oxidizing bacteria
ATP	Adenosine triphosphate
BD	Soil bulk density
BNF	Biological Nitrogen Fixation
EN	Enriched
GHG	Greenhouse gas
GWP	Global warming potentials
HSD	Honestly significant difference
NA	Natural abundance
NEE	Net ecosystem exchange
NHI	Nitrogen harvest index
<i>Ninc</i>	Nitrogen increment
PD	Particle density
PVC	Poly vinyl chloride
TN	Total N content
TP	Total soil porosity
VSWC	Volumetric soil water content
WFPS	Water-filled pore space

# 1 INTRODUCTION

## 1.1 Background

Pulse crops, also known as grain legumes, are edible legumes harvested solely for dry grain yield. They are characterized by high protein and fiber contents and low or no fat content (Tharanathan and Mahadevamma, 2003). Legumes have been an integral part of human diets since ancient times and, at present, play a key role in combating malnutrition, alleviating poverty, improving human health, increasing agricultural sustainability and ensuring food security (FAO, 2016). Canada is a major contributor to global pulse production, with pulse crop production being greatest in the prairie provinces—but mainly in Saskatchewan, which is the largest producer of peas, lentils and chickpeas (Roy et al., 2010). For example, in 2015 Saskatchewan exported 5 million tonnes of pulses with a value of nearly \$3.5 billion—accounting for approximately 80% of the Canadian total (Saskatchewan Ministry of Agriculture, 2016).

Pulse crops were once cultivated as specialty crops in cereal-based cropping systems, but as cropping systems have become more diverse, they are now routinely grown in rotation with cereal and oilseed crops (Walley et al., 2007). Including pulse crops in a rotation sequence provides a number of nitrogen and non-nitrogen based benefits (Beckie et al., 1997). A well inoculated pulse crop can fix sufficient amounts of nitrogen (N) to eliminate the need for inputs of fertilizer-N in the pulse year (Walley et al., 2007). Moreover, residual nitrogen associated with pulse crop residues can, over time, increase the nitrogen supplying power of the soil (Biederbeck et al., 1998; Beckie and Brandt, 1997; Arcand et al., 2014). In addition, the non-nitrogen benefits of including a pulse crop in cereal or oilseed based rotations include a reduction in the severity of diseases and weed infestations, breaking of pest cycles, conservation of soil moisture, and the release of nutrients other than nitrogen during decomposition of the pulse crop residues (Stevenson, 1996; Arcand et al., 2014).

Soil nitrogen is subject to a variety of microbially-driven processes—such as immobilization, mineralization and nitrification—that affect plant available forms of N. In addition, much of the

soil nitrogen used by plants is removed from cropping systems at harvesting. Soil N is also lost through processes such as denitrification, volatilization (Wrage et al., 2001) and leaching (Hedin et al., 1995). The concept of the “nitrogen cycle” is based on these processes, and explains how the nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) taken up by plants and micro-organisms is converted to organic N in living tissues and subsequently returned to soil as animal waste or senesced material where it decomposes and re-enters the cycle (Hopkins and Hüner, 1995).

Before the development of inorganic fertilizers, plants obtained all their nitrogen from the soil, the atmosphere or organic sources (e.g., animal manures and plant residues). Consequently, crop production was ultimately limited by the amount of fixed N available to the plants. However, driven by an ever-increasing world population and the accompanying need to increase crop production, this limitation was overcome by the development of synthetic inorganic N fertilizers. A key development was the introduction of the Haber-Bosch process in 1908, which facilitated the production of N fertilizers on an industrial scale. Indeed, by 2005 global demand for nitrogen fertilizers produced by the Haber-Bosch process was approximately 100 Tg (Erisman et al., 2008). At the same time, human consumption (as plant, dairy and meat products) of this nitrogen accounted for only about 17 Tg—with a large proportion of the remaining nitrogen being lost to the environment (Erisman et al., 2008). These losses can result in a variety of negative effects, such as eutrophication and acidification of terrestrial and aquatic systems and stratospheric ozone losses (Gruber and Galloway, 2008).

Of the total N lost to the environment, about 40% of fertilizer N is denitrified back to the atmosphere (Erisman et al., 2008). Nitrous oxide ( $\text{N}_2\text{O}$ ) is currently the major stratospheric ozone-depleting substance and a potent greenhouse gas, with a global warming potential that, on a mass basis, is 298 times greater than that of carbon dioxide. Synthetic nitrogen fertilizers and animal waste used in agricultural soils are the major anthropogenic sources of  $\text{N}_2\text{O}$  (IPCC, 2007; Abalos et al., 2016).

In addition to environmental issues, nitrogen fertilizer is a major economic and energy cost in continuous cropping systems (Zentner et al., 1984). Incorporation of N rich pulse residues into the soil reduces the requirement of externally added nitrogen to the subsequent crop. This, in turn, reduces the carbon footprint of the crop production process by reducing the energy consumption associated with fertilizer production. More importantly, it contributes to lowering of greenhouse

gas emissions from these cropping systems (Campbell et al., 1992). Thus, in part because of the significant environmental benefits they provide, pulse crops are considered to be crucial contributors to the sustainability of cropping systems in western Canada.

The overall objectives of my research were to examine the beneficial role of pulse crop residues in (i) improving the nitrogen supply to a subsequent cereal crop and (ii) mitigating N<sub>2</sub>O emissions relative to fertilized wheat. The specific objectives of my study were to:

- i. quantify and compare N fixation in lentil, pea, chickpea and faba bean using continuous <sup>15</sup>N<sub>2</sub> labelling;
- ii. determine the release of fixed-N into the soil of lentil, pea, chickpea and faba bean;
- iii. quantify the supply of fixed-nitrogen from the pulse crop residues to the nitrogen uptake of a subsequent crop (wheat); and
- iv. assess N<sub>2</sub>O emissions from soils containing pulse *vs.* wheat residues, and identify the sources of soil-emitted N<sub>2</sub>O.

## **1.2 Organization of the thesis**

This thesis is organized in a manuscript-based format with six chapters. A detailed review of literature (Chapter 2) will be followed by two chapters (Chapters 3 and 4) written as publishable papers. The implications of the research as a whole, together with suggestions for future research, are presented in the final synthesis chapter (Chapter 5).

Chapter 1 provides a general introduction to the thesis, addressing the research questions, objectives and the scope of study.

Chapter 2 provides a detailed review of the literature. It covers the background for soil N cycle, pulse crops, and the role of pulse crops in the sustainability of Canadian cropping systems.

Chapter 3 is the first research chapter and focuses on quantification of above- and below-ground nitrogen fixation and rhizodeposition by pulse crops using direct <sup>15</sup>N<sub>2</sub> labelling of the soil atmosphere.

Chapter 4 is the second research chapter and focuses on the contribution of the pulse residues (relative to wheat residues) to (i) the N nutrition (i.e., plant uptake) of a subsequent wheat crop and (ii) soil emitted N<sub>2</sub>O.



Chapter 5 summarizes the results and presents a synthesis of the two research chapters—including a discussion of the implications of the research and suggestions for future studies.

Chapter 6 contains a complete list of reference materials cited throughout the thesis.

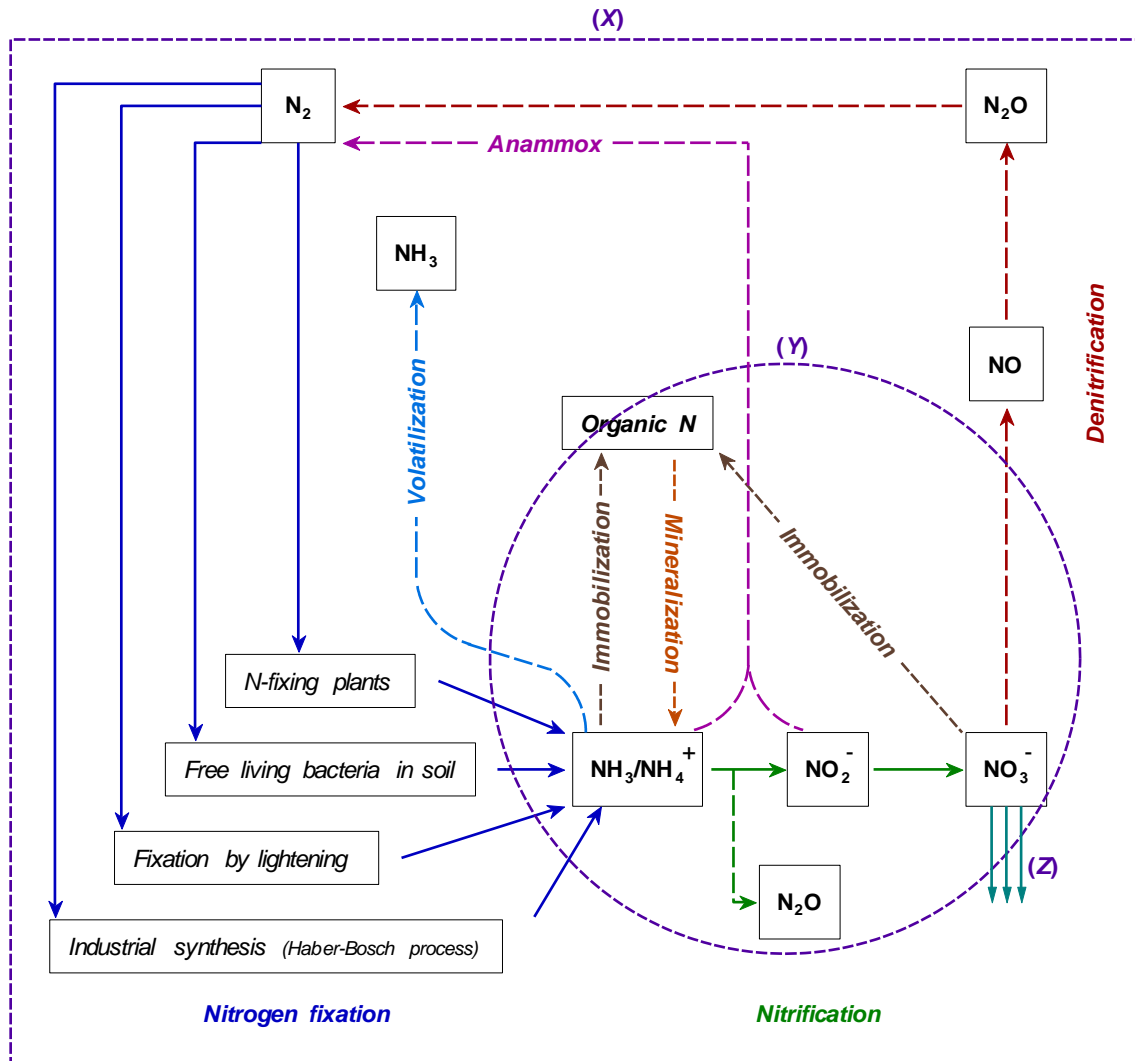
Supplemental data, figures and a detailed description of the labelling method are presented in the Appendix.

## 2 LITERATURE REVIEW

### 2.1 Nitrogen and Nitrogen Cycling

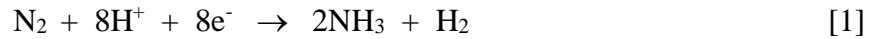
Nitrogen (N) is an essential macronutrient required by all living organisms and is generally the most limiting nutrient in agro-ecosystems. Plants have a large requirement for nitrogen, and contain more atoms of N than of any other soil-derived element, except hydrogen (Viets, 1965). Atmospheric nitrogen ( $N_2$ ) accounts for approximately 78% of the earth's atmosphere and is the ultimate source of nitrogenous compounds found in agro-ecosystems. Indeed, the fixation of atmospheric  $N_2$ , either through biological or industrial processes, is key to the nitrogen cycle (Delwiche, 1970; Akkermans and Houwers, 1983; Stein and Klotz, 2016). Once this “fixed” N enters the soil ecosystem, it undergoes a variety of microbially-mediated transformations—changing from one reactive form of N to another, and eventually being released back into the atmosphere as  $N_2$ . Rochette et al. (2006) described the N-cycle as a “loop within a loop system” in which the outer loop (Figure 2.1X) describes how  $N_2$  originating in the atmosphere moves into the soil-plant system via nitrogen fixation (industrial + biological) and is ultimately returned to the atmosphere as  $NH_3$  (volatilization), NO and  $N_2O$  (denitrification), or  $N_2$  (denitrification and anammox). The inner loop (Figure 2.1Y) describes how N moves through the soil-plant system via nitrification, immobilization, and mineralization and is lost from the system through nitrate leaching (Figure 2.1Z). The major processes involved in the nitrogen cycle are: nitrogen fixation, nitrification, mineralization, anammox, and denitrification (Figure 2.1).

Biological  $N_2$  fixation involves the reduction of atmospheric dinitrogen gas ( $N_2$ ) to ammonia ( $NH_3$ ) in the presence of the nitrogenase enzyme (Stein and Klotz, 2016), which occurs naturally in only a few species of symbiotic (*Rhizobium* and *Frankia*) and free-living (*Azospirillum* and *Azotobacter*) soil microorganisms. Prior to development of the Haber-Bosch process, which allowed for the industrial-scale production of synthetic N fertilizers, the major source of plant-available N in agro-ecosystems was biological nitrogen fixation (BNF) (Vitousek et al., 1997). Although synthetic chemical fertilizers now provide most of N used in agriculture, biologically fixed N remains an important contributor to the N fertility of annual cropping systems—accounting

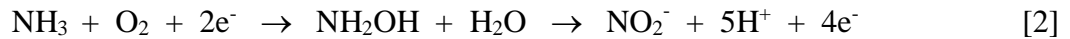


**Figure 2.1.** Schematic representation of the nitrogen cycle as a “loop within a loop” (Adapted from Rochette et al. 2006; Bernhard, 2010). The outer loop (X) describes how  $N_2$  originating in the atmosphere moves into the soil-plant system via nitrogen fixation (biological + industrial) and is ultimately returned to the atmosphere as  $NH_3$  (volatilization),  $NO$  and  $N_2O$  (denitrification), or  $N_2$  (denitrification and anammox). The inner loop (Y) describes how N moves through the soil-plant system via nitrification, immobilization, and mineralization and is lost from the system through nitrate leaching (Z).

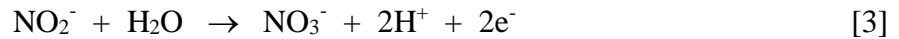
for 16% of total global (industrial + biological) N fixation (Liu et al., 2010) and contributing 50 to 70 Tg N per year (Herridge et al., 2008). Biological nitrogen fixation in soil is mediated by both symbiotic and non-symbiotic microorganisms, though the contribution of non-symbiotic (free-living) N-fixers to agro-ecosystems is generally considered to be small (Akkermans and Houwers, 1983; Gupta et al., 2006; Herridge et al., 2008). Symbiotic N fixation, on the other hand, involves plant-bacteria associations where the plant (e.g., legume) acts as host to the microorganism (microsymbiont)—providing the microorganism with carbon while receiving “fixed” N from the microorganism—and is the process that provides most of the N in natural (non-agricultural) ecosystems (Gutschick, 1981). Whereas most symbiotic associations are very specific, they involve one of three genera of bacteria (rhizobium, bradyrhizobium, and azorhizobium) that, collectively, are referred to as “rhizobia”. These bacteria infect the roots of legumes to form enlarged multicellular structures called nodules in which nitrogen fixation actively takes place via the reaction:



(Hopkins and Hüner, 1995; Bernhard, 2010). Regardless of its source, the ammonium ( $\text{NH}_4^+$ ) produced as a result of fixation is either utilized (i.e., assimilated/immobilized) by soil microorganisms and plants, or is oxidized to nitrite ( $\text{NO}_2^-$ ) and then nitrate ( $\text{NO}_3^-$ ) via nitrification. Nitrification occurs primarily under aerobic conditions (Schmidt, 1982; Bernhard, 2010) and involves two processes carried out by two groups of obligatory chemoautotrophic bacteria: (i) the oxidation of ammonia ( $\text{NH}_3$ ) to  $\text{NO}_2^-$  (via a hydroxylamine [ $\text{NH}_2\text{OH}$ ] intermediate) by ammonia-oxidizing bacteria (AOB; e.g., *Nitrosomonas*, *Nitrospira* and *Nitrosococcus*) and ammonia oxidizing archaea (AOA; e.g., *Nitrosopumilus*) via Eqn. 2:



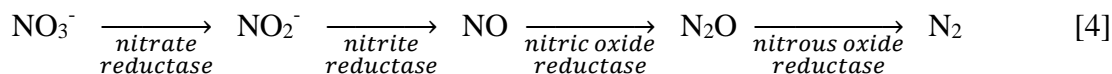
and (ii) oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  by nitrite-oxidizing bacteria (e.g., *Nitrobacter* and *Nitrococcus*) via Eqn. 3:



The resulting  $\text{NO}_3^-$  is then utilized by plants and microorganisms, which upon their death are

mineralized—thereby releasing inorganic N ( $\text{NH}_4^+$ ) back into the soil where it is recycled (Rochette et al., 2006; Bernhard, 2010; Stein and Klotz, 2016).

Under anoxic or anaerobic conditions,  $\text{NO}_3^-$  also can be consumed via denitrification, which involves the stepwise reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , nitric oxide (NO),  $\text{N}_2\text{O}$  and, ultimately,  $\text{N}_2$  (see Eqn. 4) (Poth and Focht, 1985; Hopkins and Hüner, 1995; Stein and Klotz, 2016).



Denitrifying bacteria are chemoorganotrophs and, as such, require an adequate supply of readily available organic-C as an energy source. Denitrification is inhibited by the presence of  $\text{O}_2$ , which can result in incomplete denitrification and a greater release of  $\text{N}_2\text{O}$ —a powerful greenhouse and ozone-destroying gas—into the atmosphere. In addition, anaerobic ammonium-oxidizing (anammox) bacteria can use  $\text{NO}_2^-$  as a terminal electron acceptor to oxidize  $\text{NH}_4^+$  under anaerobic conditions, though their relative contribution to N loss in agricultural soils is generally considered to be small (Long et al., 2013).

## 2.2 Factors Affecting Nitrogen Transformations in the Soil

### 2.2.1 Factors affecting biological nitrogen fixation

Biological nitrogen fixation is initiated by a signaling mechanism triggered by the plant (via root exudates) that attract specific N-fixing rhizobia, which then infect the root and initiate nodulation (Bernhard, 2012). Most farmers inoculate their fields to ensure that the most efficient strains for nodulation are present in sufficient numbers (Saskatchewan Ministry of Agriculture, 2017), even though the rhizobia strains used in commercial inoculants can survive in soil for many years (Drew et al., 2012). Apart from having a suitable microbial strain in the soil, BNF is influenced by a variety of abiotic factors such as temperature, moisture, pH and the amount of available N in the soil. For example, Houlton et al., (2008) reported that the activity of the nitrogenase enzyme (i.e.,  $\text{N}_2$  fixation) reached a maximum at  $26^\circ\text{C}$ , but was constrained at cooler temperatures by the high energy cost of  $\text{N}_2$  fixation and at warmer temperatures by depletion of available C supplies. Low temperatures also impair nodule respiration, whereas high temperatures negatively affect nodule formation (Manevar and Wollum, 1981; Hungrai and Franco, 1993; Whittington et al., 2012),

exchange of molecular signals between host plant and rhizobia (Hungria and Stacey, 1997), synthesis of leghemoglobin (Bergersen et al, 1973), and the allocation of electrons for N<sub>2</sub> reduction (Hungria and Franco, 1993). Although an analysis of the published literature by Houlton et al. (2008) suggested that there was “little evidence” for local adaptation of the nitrogenase enzyme, it is well known that certain arctic and subarctic strains of rhizobia are adapted to temperatures as low as 4°C (van Heeden et al., 2008) and that native legumes in the high arctic can fix N<sub>2</sub> at rates comparable to those of temperate legumes (including pulse crops) (Bordeleau and Prevost, 1994).

Soil water also affects BNF; e.g., water deficits reduce the number of viable rhizobia in the soil (Mulongoy, 1992) and inhibit N<sub>2</sub> fixation by reducing nodule development and the synthesis of leghemoglobin (Guerin et al., 1991; Stamford et al., 1990). Water stress also reduces the cortical permeability of nodules, which limits the supply of oxygen to the bacteria and ultimately reduces nitrogenase activity (Sprent, 1976; Durand et al, 1987; Walsh, 1995). Moreover, extreme moisture deficits can result in premature death of the nodules (Matamoros et al., 1999). Conversely, excessive soil moisture prevents the development of root hairs (the sites of nodulation) and reduces oxygen diffusion in the soil which, in turn, can adversely affect root metabolism and N<sub>2</sub> fixation (Mulongoy, 1992; Bordeleau and Prevost, 1994).

The optimum pH range for rhizobia growth is between 6 and 7 (Brockwell et al., 1991), with acidic conditions affecting the exchange of molecular signals and initial infection of the roots (Hungria and Stacey, 1997; Ferguson et al., 2013). In addition, low soil pH is often associated with Al<sup>3+</sup> and Mn<sup>2+</sup> toxicity (Graham, 1992), which creates an additional stress to rhizobia—affecting both the structure and functioning of the nodules. Similar to acidity, salinity can negatively affect rhizobia activity and nodule formation (Bruning and Rozema, 2013). Indeed, although rhizobia exhibit a wide range of salt tolerance, salinity can affect the growth and survival of rhizobia in the soil, restrict root colonization, inhibit root infection and nodule development, and/or impair active nodule functioning (Abdelmoumen et al., 1999).

Biological nitrogen fixation is also influenced by soil nutrient levels; e.g., high soil nitrogen levels inhibit BNF (Somesegaran et al., 1990; Peoples and Crasswell, 1992; Walsh and Carroll, 1992; Viosin et al., 2002; Bhatia et al., 2001). More specifically, it is observed that high levels of available N reduce the number and mass of the nodules, as well as nitrogenase activity, in pulse crops (Leidi and Rodriguez-Navarro, 2000). Conversely, N<sub>2</sub> fixation is inhibited by low levels of

available soil P (Hussain, 2017)—largely reflecting the fact that BNF is an energy-intensive process that requires large amounts of adenosine triphosphate (ATP) to proceed. Indeed, 16 molecules of ATP are required for each molecule of  $N_2$  reduced (Berg et al., 2002); consequently, P deficiencies can result in low levels of root ATP which, in turn, reduces  $N_2$  fixation. Not surprisingly, therefore, it has been suggested that the P response of legumes is often a reflection of improved  $N_2$  fixing activity (Singleton et al., 1985).

### **2.2.2 Factors affecting nitrification**

Nitrification involves the microbially-mediated oxidation of reduced forms of N ( $NH_3$  or  $NH_4^+$ ) to  $NO_3^-$  (see the inner loop [X] in Fig. 2.1). Ammonia oxidation (Eqn. 2) is the rate-limiting step in the process and is carried out by microorganisms possessing the gene (*amoA*) encoding for the production of ammonia monooxygenase. Nitrite oxidation (Eqn. 3) is carried out by microorganisms capable of producing nitrite oxidoreductase, with oxygen supplied by water (Prosser, 2012). Consequently, interactions between soil moisture and aeration have a significant impact on nitrification (Sahrawat, 2008). Indeed, soil water content regulates the accessibility of both substrate ( $NH_4^+$  and  $NO_2^-$ ) and  $O_2$  to the microbes responsible for nitrification (Norton and Stark, 2011; Schimel et al., 2007). For example, under dry conditions, the water films lining soil pores become thin, thereby restricting the mobility of both substrates and nitrifiers (Stark and Firestone, 1995; Olivera et al., 2014). Conversely, these films thicken under wet conditions, which restricts  $O_2$  diffusion within the soil (Norton and Stark, 2011) and inhibits nitrification.

Nitrification also is influenced by soil pH (Cookson et al., 2007; Tian et al., 2012; O'Sullivan et al., 2013) and salinity (Noe et al., 2013). Although nitrification in soils can occur over a relatively wide pH range, the optimal pH has been estimated at between 6.5 and 8.5 (Sahrawat, 2008). At low pH (<6), ammonia oxidation is inhibited, while at high pH (>8) it is the oxidation of nitrite to nitrate that is reduced. Nitrification also decreases as salinity increases (Akhtar et al., 2012), though this effect is generally regarded as reflecting the effect of salinity on overall microbial metabolism (Ward, 2013).

### **2.2.3 Factors affecting denitrification**

Denitrification occurs as four enzymatically catalyzed reductive reactions regulated by denitrifying bacteria (see Fig. 2.1, outer loop). However, most denitrifiers do not possess the complete set of denitrifying enzymes, and thus carry out only a part of the process (Zumft, 1997).

Apart from the composition of the microbial community, and the presence of sufficient quantities of a suitable substrate (i.e.,  $\text{NO}_3^-$ ), the factors known to have a major influence on denitrification are: (i)  $\text{O}_2$  availability—generally reflecting the effects of soil water content; (ii) soil pH—generally between 4 and 8; and (iii) soil temperature—generally  $\geq 5^\circ\text{C}$  (Bremner and Shaw, 1958; Firestone, 1982; Rhein-Baben, 1990; Butterbach-Bahl et al., 2013). The  $\text{O}_2$  status of the soil is determined by the rates of consumption and re-supply which, in turn, are controlled by microbial activity and soil water content. In soils, denitrification is most often limited by  $\text{O}_2$  availability—with  $\text{O}_2$  inhibiting both the synthesis and activity of denitrifying enzymes (Knowles, 1982)—which, in turn, is largely controlled by soil moisture. However, denitrification is also known to occur in temporary anaerobic microsites in the soil, mediated by facultative anaerobes (Tiedje et al., 1984).

Bremner and Shaw (1958) first noted that denitrification was initiated at a water content equivalent to 60% water-filled pore space (WFPS), while Davidson et al. (2000) report that  $\text{N}_2\text{O}$  production/emission is optimal at 70–80% WFPS. At higher soil moisture contents, complete denitrification is favored as  $\text{N}_2\text{O}$  is reduced to  $\text{N}_2$  (Weier et al., 1993; Butterbach-Bahl et al., 2013). The production/emission of  $\text{N}_2\text{O}$  at low soil water contents (i.e., 35–60% WFPS), on the other hand, is primarily a result of nitrification (Robertson and Tiedje, 1987; Bateman and Baggs, 2005).

Denitrification, like all microbially-mediated processes, is temperature sensitive and rates of denitrification increase rapidly as the temperature increases from  $2^\circ\text{C}$  to  $25^\circ\text{C}$  (Bremner and Shaw, 1958). However, many denitrifying microorganisms are active at low (near  $0^\circ\text{C}$ ) temperatures and  $\text{N}_2\text{O}$  emissions associated with freeze/thaw events (such as at snow-melt in the spring) can account for large proportions of the total annual  $\text{N}_2\text{O}$  budget (Groffman et al., 2009; Wagner-Riddle et al., 2017). Denitrification also is influenced by soil pH, with a pH around 8 facilitating high rates of denitrification and inhibition occurring under acidic conditions (Simek and Cooper, 2002; Liu et al., 2013). Soil pH is known to influence the denitrifier community composition (Brenzinger et al., 2015), which in turn affects denitrification,  $\text{N}_2\text{O}$  emissions, and the  $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$  ratio (Cuhel et al., 2010). Indeed, Cuhel et al. (2010) reported that although soil pH had no effect on total denitrification, the  $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$  ratio increased with decreasing pH.



### 2.3 Pulse crops of Significance in Western Canada

Plants are an essential source of proteins and are consumed widely in developing countries where meat and other livestock products are too expensive for routine consumption. Grain legumes (i.e., pulses) fit into this need as they can provide a significant amount of dietary protein (Christou, 1997). In developed countries, on the other hand, pulses are more important as a source of fiber. Indeed, dietary fibers are known to provide a number of health benefits, such as reducing the risk of cardio-vascular diseases (Riccioni et al., 2012). As a result of these benefits, pulses play a vital role in enhancing human health and ensuring food security. In addition to health benefits, growing pulses in rotation with cereal and oilseed crops can contribute to the long-term sustainability of agricultural systems, enhance soil health, and conserve soil biodiversity.

Pulse crops have been an integral part of Canadian cropping systems for several decades (Walley et al., 2007) with pulse production centered mainly in Saskatchewan (SPG, 2017). The major pulse crops grown in Saskatchewan are chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), dried pea (*Pisum sativum*) and broad bean (*Vicia faba*). This reflects that (i) there is a strong and growing demand for these pulses in the global market (Statistics Canada, 2016) and (ii) under the climatic conditions characteristic of the Canadian prairie provinces, these pulses are high yielders that can be successfully integrated into crop rotations (Suryapani et al., 2012; Beckie and Brandt, 1997; Li et al., 2011). Moreover, when inoculated with the appropriate rhizobium species, pulse crops can fix approximately 50 to 80% of their N from the atmosphere (Walley et al., 2007)—reducing both input and environmental costs during the pulse year and beyond.

Pea is one of the most widely cultivated pulse crops in all agro-ecological zones of Saskatchewan. In 2016, approximately two million acres of land was seeded with pea, and the five-year average yield was 2,310 kg ha<sup>-1</sup> (Saskatchewan Ministry of Agriculture, 2017). Pea can fix up to 80% of its nitrogen requirement when properly inoculated with the appropriate rhizobium strain (*R. leguminosarum*). As a result, the amount of fixed nitrogen in pea is reported to be between 56 and 168 kg per ha (Ruisi et al., 2012; Saskatchewan Ministry of Agriculture, 2017). The N-fixing capacity of pea can be enhanced by dual inoculation with arbuscular mycorrhizal fungi (Xavier and Germida, 2003), and BNF in pea is greater when cultivated with more diverse crop rotations (Knight, 2012). In addition, pea stubble can provide additional nitrogen to subsequent crops which can result in high yields under conditions of low soil fertility (Adderley et al., 2004; Beckie et al.,

1997).

Lentil is another important pulse crop grown in Saskatchewan. In 2016, five million acres of land was seeded to lentil and the five-year average yield of lentils in the Province was 1,660 kg ha<sup>-1</sup>. There are several different varieties of lentil grown in Saskatchewan; the main varieties being green and red lentil, with a few specialty varieties grown in small volumes (Saskatchewan Ministry of Agriculture, 2017). Under proper inoculation lentil can fix 70 to 80% of its nitrogen through BNF (Kurdali et al., 1997; van Kessel, 1994; Bremer et al., 1990), and the amount of fixed N in lentil is reported to be between 34 and 134 kg ha<sup>-1</sup> (Saskatchewan Ministry of Agriculture, 2017). Nitrogen benefits from lentil are reported to be higher under diverse cropping systems (Matus et al., 1996).

Chickpea is another important pulse crop for which there is a growing demand both as a protein source and as a health food in both international and local markets. Commercial cultivation of chickpea in Saskatchewan started in the mid 1990's and there are two major chickpea varieties grown; i.e., 'Desi' and 'Kabuli'. In 2016, the area seeded to chickpea in the Province was reported to be approximately 65,000 ha, and the five-year average yield for the Province was 1964 kg ha<sup>-1</sup> (Saskatchewan Ministry of Agriculture, 2017). The rhizobium species used to inoculate chickpea (*Mesorhizobium ciceri*) differs from the strain used for pea, lentil and faba bean (*R. leguminosarum*) (Saskatchewan Ministry of Agriculture, 2017). Nevertheless, when properly inoculated, chickpea can fix 60 to 80% of its nitrogen through BNF (Agriculture and Agri-Food Canada, 2015).

Faba bean (broad bean) is cultivated for both human consumption and as a livestock feed. Faba bean varieties are classified by their tannin levels. Zero tannin varieties (e.g., Snowbird and CDC Snowdrop) used for green manure and silage production, are widely cultivated in Saskatchewan. In 2016, area seeded to faba bean in the Province was reported to be approximately 20,000 ha (Saskatchewan Ministry of Agriculture, 2017). Among the four major pulse crops grown in Saskatchewan, faba bean has the ability to fix greatest amount of nitrogen through BNF (Walley et al., 2007); i.e., faba bean is reported to meet 80 to 90% of its N requirement via BNF (Walley et al., 2007), and fix 90 to 180 kg N ha<sup>-1</sup> (Saskatchewan Ministry of Agriculture, 2017).

## 2.4 Rotational Benefits of Pulse Crops

Inclusion of a grain legume in crop rotations has been shown to increase the yield of the subsequent crop compared to monocultures (Wright, 1990; Rowland et al., 1994; Stevenson and van Kessel, 1996; Beckie and Brandt, 1997; Gan et al., 2001; Soon and Clayton, 2002; Soon and Arshad, 2002; Miller et al., 2003; Gan et al., 2003; Jenson et al., 2009; Chen et al., 2012; Luce et al., 2015; Lupwayi and Soon, 2016). Whereas, in part, this yield increase reflects an increase in N availability to the subsequent crop due to the high N content of the pulse crop residues, it is often observed to exceed that which could be expected solely on the basis of the added N. Indeed, the rotation benefits of pulse crops can be grouped into two categories: nitrogen and non-nitrogen benefits (Stevenson and van Kessel, 1996; Beckie and Brandt, 1997).

### 2.4.1 Nitrogen benefits

As with all legumes, pulse crops are capable of biological nitrogen fixation by forming a symbiotic relationship with a *Rhizobium* species such as *R. leguminosarum* (Peoples et al., 1995; Howieson et al., 2008), which allows them to reduce their requirement for synthetic N fertilizer (Zentner et al., 1984) while also providing an “N benefit” to the following year’s crop (Walley et al., 2007). The concept of a “nitrogen increment” (*Ninc*)—defined as the difference between the nitrogen derived from atmosphere (%Ndfa) and the nitrogen harvest index (NHI) (Walley et al., 2007)—is important when evaluating the contribution of pulse crop residues to soil nitrogen pools. Indeed, pulse crops provide a positive *Ninc* only when the amount of fixed-N in the residues exceeds the amount exported with the grain (Toomsan et al., 1995; Crozat and Fustec, 2006; Walley et al., 2007). For example, the contribution of pea residue to soil N pools decreases as the NHI increases (Senaratne and Hardarson 1989; Evan et al., 1989; Jensen 1989). Moreover, Walley et al. (2007) reported that in the Northern Great Plains, pulse crops need to obtain an average of 48% or more of their N requirement through fixation in order to contribute to soil nitrogen pools. Field pea, for example, generally fixes about 1.5-times more N than it requires (Bremer et al., 1988; Cowell et al., 1989; Androsoff et al., 1995), thus yielding a positive *Ninc*. Likewise, lentil and faba bean usually contribute to a positive N increment, while chickpea is unlikely to do so (van Kessel and Hartley, 2000; Walley et al., 2007). In general, BNF by pulse crops is inversely related to the amount of available N in the soil (Kiers et al., 2003; Gan et al., 2004; Salvagiotti et al., 2008; Schipanski et al., 2010; Hossain et al., 2016), though certain pulses—such as faba bean—have the

ability to maintain high rates of BNF even when levels of available N in the soil are high (Hossain et al., 2016).

The net benefit of a positive *Ninc* is that, to produce a given yield, the amount of fertilizer-N required for a cereal or oilseed crop grown on pulse residues is less than that of the same crop grown on non-pulse residue (Beckie and Brandt, 1997). Thus, including a pulse in rotation on a low fertility soil can improve the economic return for the subsequent cereal crop by reducing the fertilizer-N input required to grow the cereal. For example, Adderley et al., (2006) found that under low soil fertility conditions cereals grown on pea residue produced similar yields to those obtained at high levels of available soil N. This presumably reflects a greater mineralization of the pea residue during the wheat phase of the cropping sequence. Indeed, Gan et al. (2010) reported that N mineralization of pea and lentil residues in soil was 19% greater than that of wheat residues. The net result is that a positive *Ninc* can reduce input costs and energy requirements of cropping systems (Burgess et al., 2012) by decreasing the demand for external applications of nitrogen fertilizer (Beckie and Brandt, 1997; Peoples et al., 2009; Arcand et al., 2014). The quantity of N fixed by a pulse crop—and hence returned to the soil—is determined by crop genotype and the presence of an effective plant-rhizobium symbiosis, as modified by interactions between the legume and the soil N environment (Unkovich and Pate, 2000). However, even if a pulse crop fails to generate a positive *Ninc*, it can still provide indirect N benefits by preserving soil available N (i.e., N sparing effect). That is, by reducing the amount of N uptake from the soil available-N pool due to their fixing ability and thus retaining high post-harvest N levels in soil (Maidl et al., 1996).

Rhizodeposition is another process that contributes to soil inorganic N pools—especially for pulse crops. Plant roots release organic and inorganic N due to exudation and turnover of root tissue in the rhizosphere (Wichern et al., 2008), and N derived from rhizodeposition can vary from 7% to 57% in mature plants (Fustec, 2010). The main pathways of rhizodeposition are: (i) senescence; i.e., the death and decay of roots and nodules; (ii) exudation of soluble compounds; (iii) the sloughing off of root boarder cells; and (iv) secretion of mucilage (Fustec et al., 2010). Of these, the death and decomposition of roots and root nodules is the major pathway by which fixed N enters the soil N pool (Dubach and Russelle, 1994). For legumes, including pulse crops, this N is mainly in the form of  $\text{NH}_4^+$ , ureides, and amino acids—including glycine and serine, which are recovered in high concentrations in root exudates, and asparagine and glutamine, which are present in high concentrations in root extracts (Brophy and Heichel, 1989; Paynel and Cliquet, 2003;

Lesuffleur et al., 2007).

#### **2.4.2 Non-nitrogen benefits**

The non-nitrogen benefits of including a pulse crop in rotations include: restricting root and shoot diseases (Nayyer et al., 2009), reducing weed growth (Stevenson and van Kessel., 1996), increasing nutrient and organic matter availability (Barber, 1995; Grant et al., 2002), regulating nutrient cycling, sequestering carbon (Batterman et al., 2013), and reducing greenhouse gas emissions (Lemke et al., 2007; Wrage et al., 2001; IPCC, 2006). The inclusion of pulse crops in rotations with cereal and oilseed crops also helps to maintain soil biodiversity and enhance soil quality and health (Wagg et al., 2015). For example, pulse crops form a strong symbiotic relationship with arbuscular mycorrhizal fungi which, in turn, secrete soil proteins that enhance aggregate stability and improve soil structure (Campbell et al., 1993; Sultani et al., 2007; Gould et al., 2016). In general, root exudates stimulate microbial activity in the rhizosphere and provide beneficial compounds for plant growth (Bullock, 1992; Smiley et al., 1991; Bashan and de-Bashan, 2005). In the case of pulse crops, N<sub>2</sub> fixation is initiated by the release of chemical signals (flavonoids) from the plant roots that attract the rhizobia to the roots and induce transcription of genes responsible for the production of the Nod factors that initiate nodule formation (Phillips and Tsai, 1992; Liu and Murray, 2016). The production of hydrogen (H<sub>2</sub>) as a by-product of BNF also affects the microbial composition of the soil by stimulating the growth of H<sub>2</sub>-oxidizing, plant growth-promoting rhizobacteria (Golding et al., 2012; Angus et al., 2015). As well, it has been suggested that decomposing pulse crop residues release growth-promoting substances that benefit the subsequent crop (Ries et al., 1977; Stevenson and van Kessel 1996; Lupwayi and Kennedy, 2007; Arcand et al., 2014). Soil microbial communities also are affected by the N released during decomposition of pulse residues (Lupwayi et al., 2004; Partey et al., 2014). Overall, it is due to their ability to provide both nitrogen and non-nitrogen benefits that pulses are frequently included in rotation with cereal and oilseed crops (Walley et al., 2007).

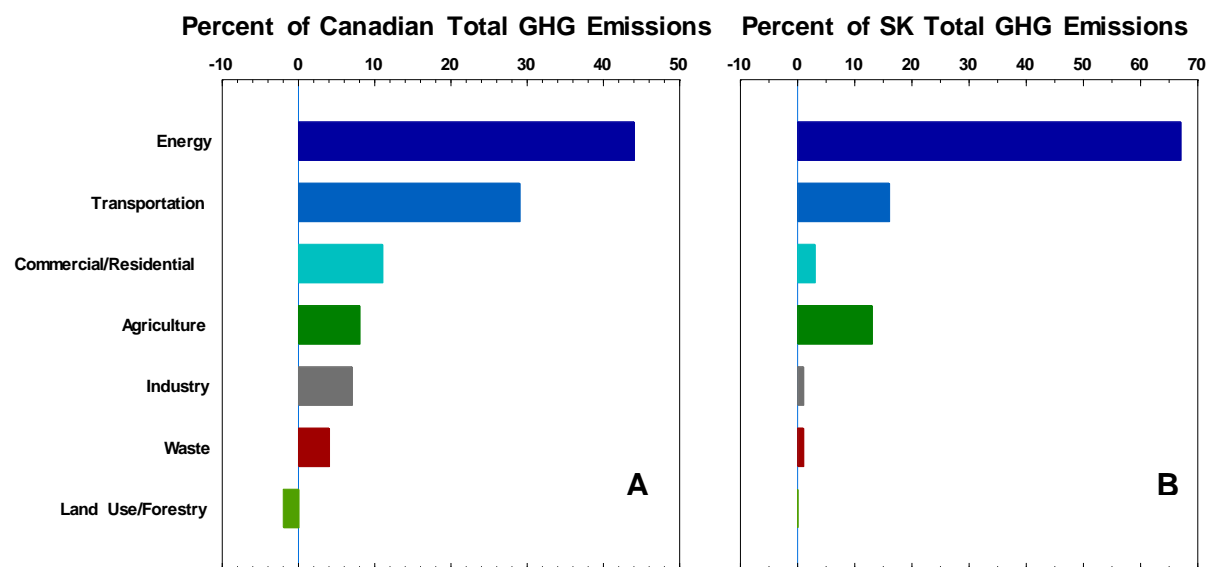
Compared to monocropping, rotational cropping systems are known to disrupt the life cycle of crop-specific pathogens (Kirkegaard et al., 2008); thus, providing a yield advantage to subsequent crops in the rotation. For example, in a study to assess the “rotation benefit” of including a pulse crop (pea [*Pisum sativum* L.]) in rotation with wheat, Stevenson and van Kessel (1996) found that more than 90% of the rotation benefit was related to non-N benefits that included disease

suppression. Peoples et al., (2009) also observed that the cultivation of grain legumes inhibited soil-borne pathogens of cereal crops. Rotational cropping that includes grain legumes also has been shown to improve soil properties such as aggregate stability (Blair and Crocker, 2000), soil organic carbon retention (Hajduk et al., 2015), bulk density, porosity (Chan and Heenan, 1996; Hubbard et al., 2013), soil water retention (Hubbard et al., 2013), and water infiltration (Kemper et al., 2011). For example, Gan et al., (2003) speculated that improved soil moisture content was a factor in the increased grain yields and grain crude protein concentrations observed for wheat grown after chickpea, lentil, or dry pea. Likewise, faba bean, which has a deep rooting system that allows it to access water in deeper soil layers—especially in water-stressed environments—while using less water from the shallower depths; may increase the water available to more shallow rooting crops (such as wheat) grown in rotation (Stutzel and Kage, 1998; Schmidtke, 2007; Lopez-Bellido et al., 2007). It is important to note, however, that these soil properties—and the rotational effects on these properties—also are impacted by management and environmental factors (Ball et al., 2005)—and that the effects of pulse crop residues on these soil properties are not always positive. For example, whereas Lafond et al. (2005) reported that the water-use efficiency of wheat grown on pea stubble was 10% greater than that of wheat grown on wheat stubble, Chan and Heenan (1996) reported that the beneficial effects of crop residue on soil structure and water stability were greater for canola than for field pea.

## **2.5 Agriculture Greenhouse Gas Emissions**

Greenhouse gases (i.e., CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, and fluorinated gases) absorb infrared radiation in the atmosphere—trapping heat and warming the earth’s surface—consequently, they are key contributors to climate change. The primary sources of these GHGs are: energy production; transportation; industry; commercial and residential buildings; agriculture; and land use, land use change, and forestry; and water and wastewater (IPCC, 2004). Although carbon dioxide (CO<sub>2</sub>) is the major contributor to total GHG emissions, N<sub>2</sub>O and methane (CH<sub>4</sub>) are the primary GHGs associated with the agriculture sector (Snyder et al., 2009), which in large part reflects their much greater global warming potentials (GWP). That is, CH<sub>4</sub> and N<sub>2</sub>O have GWPs of 25 and 298, respectively—indicating that 1-ton of CH<sub>4</sub> or N<sub>2</sub>O released into the atmosphere will absorb 25- and 298-times more heat energy than 1-ton of CO<sub>2</sub> (IPCC, 2007).

In Canada, the agriculture sector accounts for only about 8% of total GHG emissions (Fig. 2.2A), but 26% of total CH<sub>4</sub> and 73% of total N<sub>2</sub>O emissions (Environment Canada, 2015). In Saskatchewan, however, the contribution of the agriculture sector is even larger, accounting for 17% of total GHG emissions (Fig. 2.2B), but 29% and 84% of total CH<sub>4</sub>, and N<sub>2</sub>O emissions, respectively. Total GHG emissions associated with the agriculture sector reached a peak of 62 Mt CO<sub>2</sub>-eq in 2005, but have been trending downward since that time (Environment Canada, 2015). However, this trend largely reflects a decrease in the number of livestock in Canada, while N<sub>2</sub>O emissions from agricultural soils have increased by about 26% (i.e., from 19 to 24 Mt CO<sub>2</sub>-eq) during the same period.



**Figure 2.2. Sector contributions to the 2013 Canadian national (A) and Saskatchewan provincial (B) greenhouse gas (GHG) inventory. Emission data are on a CO<sub>2</sub>-equivalent basis and were obtained from the National Inventory Report (1990–2013): Greenhouse Gas Sources and Sinks in Canada, Part 3 (Environment Canada, 2016).**

### 2.5.1 Soil-derived greenhouse gases

Soils are both a source and a sink of greenhouse gases and, as such, play a key role in climate change research (Oertel et al., 2016). Soil-derived CO<sub>2</sub> is produced via respiration and includes contributions from both microbial (heterotrophic) and root (autotrophic) respiration (Beverly and Franklin, 2015). Agricultural soils are a sink for CO<sub>2</sub> when photosynthetic CO<sub>2</sub> uptake by the crop exceeds soil (+ plant) respiration; i.e., when the net ecosystem exchange (NEE) is negative.

Conversely, soils are a net source of CO<sub>2</sub> when the NEE is positive. In Canada, CO<sub>2</sub> emissions from agricultural soils decreased from 7.7 million tonnes in 1981 to 1.8 million tonnes in 1996 (Desjardins, 1998)—mainly due to the adoption of no-till agriculture—and today agricultural soils are generally believed to be “carbon sinks”.

Methane is both produced (under anaerobic conditions) and consumed (under aerobic conditions) by soil microorganisms. Consequently, whether soils are a net source or sink for CH<sub>4</sub> depends on the dynamic equilibrium between production (methanogenesis) and consumption (methanotrophy) (Lemke et al., 2010). For example, in semiarid regions with well-aerated soils—conditions typical of the western Canadian prairie provinces—agricultural soils tend to be small sinks for CH<sub>4</sub> (Mosier et al. 2006; David, 2014).

As discussed in Section 2.1, soil-derived N<sub>2</sub>O is produced through both nitrification (under aerobic conditions) and denitrification (under anoxic or anaerobic conditions). Moreover, N inputs in the form of fertilizers, manures and crop residues are widely recognized as primary sources of the N<sub>2</sub>O emitted from agricultural soils; i.e., from croplands and fertilized/grazed pastures (Reay et al., 2012). Although soils are often observed to uptake small amounts of N<sub>2</sub>O from the atmosphere (Chapuis-Lardy et al., 2007; Liebig et al., 2010)—and despite recent reports of a previously unknown group of N<sub>2</sub>O-reducers (Jones et al., 2013)—the sink potential of soils is thought to be quite small (Schlesinger, 2013).

In well-aerated soils, such as those characteristic of the semi-arid prairies of western Canada, the overall sink potential of agricultural soils is largely determined by the balance between NEE and N<sub>2</sub>O production/emission. Indeed, efforts to enhance C-sequestration are often negated by a concomitant increase in N<sub>2</sub>O emissions (Smith, 2010). That is, because of its large GWP, releases of even small amounts of N<sub>2</sub>O into the atmosphere can offset fairly large amounts of CO<sub>2</sub> uptake; e.g., it takes only 3.35 kg of N<sub>2</sub>O to offset 1 tonne of CO<sub>2</sub> removed from the atmosphere. As a result, there is considerable interest in reducing N<sub>2</sub>O emissions from agricultural soils.

### **2.5.2 Nitrous oxide emissions from cropped soils**

It is well known that N<sub>2</sub>O emissions from soils are characterized by a high degree of both spatial and temporal variation (Yates et al., 2006; Groffman et al., 2009; Hénault et al., 2012) that largely reflects the occurrence of both “hot spots” (locations with much higher emission rates than the surrounding area) and “hot moments” (short periods of time with emission rates much greater than



the long-term average) of increased emissions. Moreover, the spatial and temporal components of both intra- and inter-annual variability are driven largely by small-scale (and short-term) changes in soil properties, crop cover, and nutrient (particularly N) availability (Butterbach-Bahl et al., 2013). Nitrous oxide emissions from soils also are known to be significantly affected by the timing and intensity of precipitation events. Indeed, emission intensity generally follows what has been described as an event based/background pattern wherein the most intense emissions are associated with some sort of triggering event (Yates et al., 2006). The primary triggering events being snow melt accompanying an increase in temperature during the spring thaw; precipitation events following the application of fertilizer N; and large, early- to mid-season rainfall events that can trigger denitrification of plant available N in the soil. In general, late-season precipitation events do not trigger large N<sub>2</sub>O emissions as, by then, the available N pool has been largely depleted by the standing crop. One result of this variability is that annual N<sub>2</sub>O budgets are usually associated with a high degree of uncertainty that can make it difficult to detect treatment-induced differences, and thus to identify mitigation options.

Apart from edaphic and environmental factors, management-related factors such as crop type; fertilizer source, rate, timing and placement; irrigation; and tillage and crop residue management also affect N<sub>2</sub>O emissions from soil (Williams et al., 1992; Snyder et al., 2009). In general, N<sub>2</sub>O emission intensity reflects the interaction of several factors. For example, fertilizer-N source effects are often confounded by placement (e.g., broadcast vs. incorporated, seed-placed vs. banded) (Engel et al., 2010; Halvorson and Del Grasso, 2013). In addition, Venterea et al. (2005) found that N<sub>2</sub>O emissions associated with broadcast urea were greater under no-till and conservation tillage compared to conventional tillage; while for anhydrous ammonia, emissions were greater under conventional tillage than under no-till or conservation tillage. In a study of farming systems across the different ecoregions of Canada, Helgason et al., (2005) observed that no-till systems tended to increase N<sub>2</sub>O emissions in humid regions while reducing emissions in arid regions.

Nitrous oxide emissions also are influenced by the timing of the N fertilizer application (Hultgreen and Leduc, 2003; Burton et al., 2010; Liebig et al., 2010). For example, Burton et al. (2010) demonstrated that a split-application of N fertilizer was an effective strategy for reducing N<sub>2</sub>O emissions in potato production, though this effect was strongly influenced by the amount and timing of precipitation at the time of the second application. Whereas similar results were reported

by Venterea and Coulter (2015) for corn production in Minnesota, Wang et al. (2015) reported a significant decrease in N<sub>2</sub>O emissions associated with a split N application for corn production in the semi-arid Loess Plateau in China. The potential for N<sub>2</sub>O emissions also is greater following a fall N application compared to a spring application, as greater quantities of soil nitrogen are available for denitrification during the peak emission period that accompanies snowmelt. Indeed, large N<sub>2</sub>O emissions have been observed during spring thaw following a fall N application in both dryland (Nyborg et al., 1997; Lemke et al., 1998; Liebig et al., 2010) and irrigated cropping systems (Mosier et al., 2006; Sainju et al., 2012).

### **2.5.3 Greenhouse gas benefits of including a pulse crop in rotation**

The inclusion of pulse crops in rotations with cereals and oilseeds is becoming increasingly popular in dryland production regions such as the semi-arid prairies of western Canada (MacWilliams et al., 2014; Gan et al., 2015). Pulses (specifically, short-season varieties) are valued for their low requirement for N fertilizer and their contribution to soil water conservation, as well as for the N and non-N benefits (see Section 2.4) they provide to subsequent crops in rotation (Jensen et al., 2012; Gan et al., 2015). In terms of N<sub>2</sub>O, cumulative emissions during the pulse phase of a rotation are generally quite low compared to those from a cereal or oilseed phase of the rotation (Lemke et al., 2002; Rochette et al., 2004; Zong et al., 2004; Helgason et al., 2005). Moreover, Jeuffroy et al. (2013) estimated that including a pea crop in rotation with wheat and canola could reduce the cumulative (i.e., 3-yr total) N<sub>2</sub>O emissions of the rotation by 20–25%. They attributed this primarily to the replacement of fertilizer-N with biologically fixed N, thus reducing the amount of NO<sub>3</sub><sup>-</sup> in the soil that was available for denitrification.

In addition to N fertilizer, crop residues can be a significant source of the N emitted as N<sub>2</sub>O from agricultural soils (IPCC, 2006; Delgado et al., 2010). Indeed, the IPCC (2006) estimates that 17% of all agriculture-related N<sub>2</sub>O emissions are associated with the decomposition of crop residues. Moreover, at present, the IPCC applies the same emission factor to crop residues as it does to synthetic N fertilizers (Environment Canada, 2015). This prompted Delgado et al., (2010) to examine the contribution of crop residues to N<sub>2</sub>O emissions using a <sup>15</sup>N-labelling technique. Using this technique, they demonstrated that the potential for N<sub>2</sub>O emissions from crop residues was about 60% less than that from synthetic N-fertilizers. Conversely, recent meta-analyses examining the effects of crop residue additions on N<sub>2</sub>O emissions from agricultural soils (Chen et al., 2013;

Shan and Yan, 2013) concluded that N<sub>2</sub>O emissions associated with crop residue additions were generally comparable to, or greater than, those associated with synthetic N-fertilizers (i.e., when applied at equivalent N rates). These meta-analyses also found that the effect of crop residues on N<sub>2</sub>O emissions tended to decrease with increasing C:N ratio. Indeed, at intermediate (25-75) and high (>75) C:N ratios, available soil N is immobilized and sequestered in soil organic matter (Huang et al., 2004; Delgado et al., 2010). Conversely, residues with a low C:N ratio provide the readily available C and N that is necessary for both nitrification and denitrification, and which contributes to high N<sub>2</sub>O emissions (Baggs et al., 2000; Huang et al., 2004).

Pulse crop residues tend to have low C:N ratios—relative to cereal and oilseed residues (Gan et al., 2011)—and, consequently, could result in increased N<sub>2</sub>O emissions during the year following the pulse phase of a rotation. However, factors other than N availability also are known to affect residue decomposition (Trinsoutrot et al., 2000; Frimpong and Baggs, 2010; Badagliacca et al., 2017) and, hence, the magnitude and direction of N<sub>2</sub>O emissions associated with crop residue additions. This implies that residue quality is perhaps an even more important factor than residue quantity in the regulation of N<sub>2</sub>O emissions. Moreover, Badagliacca et al. (2017) found that the magnitude of the N<sub>2</sub>O emissions associated with crop residue decomposition was strongly influenced by soil type (e.g., texture, pH, inherent N fertility). For example, N<sub>2</sub>O emissions associated with faba bean residue were greater than those associated with wheat residue in a low fertility, high pH soil; while the opposite was true in a high fertility, low pH soil.

It is also worth noting that interactions between crop residues and applied fertilizer-N can significantly impact the magnitude of soil-derived N<sub>2</sub>O emissions (Baggs et al., 2003; Frimpong and Baggs, 2010). For example, Baggs et al. (2003) observed that the proportion of N<sub>2</sub>O-N derived from fertilizer decreased when the crop residues were incorporated into the soil compared to when they were surface mulched. At the same time, they reported that the magnitude and direction of N<sub>2</sub>O emissions associated with the combined application of NH<sub>4</sub>NO<sub>3</sub> fertilizer and bean (*Vicia faba*) residue was influenced by tillage treatment—with an increase in emissions under zero-till and a decrease in emissions under conventional tillage. However, regardless of whether N<sub>2</sub>O emissions associated with the decomposition of pulse residues are greater or lesser than those associated with cereal or oilseed residues, the overall contribution of pulse crops to cumulative (rotation-level) emissions is generally small (Peyrard et al., 2016). Indeed, when compared with

continuous cropping systems, rotations that include a legume (including grain legumes) emit less total N<sub>2</sub>O (Robertson et al., 2000; Mosier et al., 2006; Barton et al., 2010; Officer et al., 2015).

## **2.6 Tracking the Fate of Fixed Nitrogen in Soil-Plant Systems**

Although studies of (i) BNF by pulse crops and (ii) the fate of the N in pulse residues are not uncommon (Carranca et al., 1999; Soon and Arshad, 2004; Lupway and Kennedy, 2007; Peoples et al., 2009; Gan et al., 2010; Jenson et al., 2010;), there have been only a few studies—either in the greenhouse or the field—that have addressed both of these issues (Delgado, 2010; Delgado et al., 2010; Chen et al., 2013; Shan and Yan, 2013). One of the major reasons for this is the difficulty (and expense) associated with measuring small (ppb or ppm) changes in plant and soil N pools against a relatively large (%N) background (Unkovich et al., 2008). Nitrogen has two naturally-occurring stable (non-radioactive) isotopes: nitrogen-14 (<sup>14</sup>N) and nitrogen-15 (<sup>15</sup>N)—with atmospheric N<sub>2</sub> containing only 0.3663 atom% <sup>15</sup>N (Mariotti, 1983). Consequently, agricultural (and ecological) studies frequently use <sup>15</sup>N as a tracer for plant and soil nitrogen (Knowles and Blackburn, 1993). Broadly speaking, these methods can be grouped into two categories: natural abundance (NA) methods and <sup>15</sup>N-enrichment methods.

Whereas <sup>15</sup>N-enrichment methods rely on the addition of <sup>15</sup>N-enriched materials to the system, NA methods rely on measurements of very small, but naturally occurring, changes in the <sup>15</sup>N/<sup>14</sup>N ratio (i.e., δ<sup>15</sup>N) in a system (Bedard-Haughn et al., 2003). Natural abundance methods are frequently used to quantify BNF in legumes—including grain legumes—both in the greenhouse and in the field (Unkovich et al., 2008). Although the <sup>15</sup>N NA method has a number of advantages (e.g., it involves less disturbance, is less costly, and enjoys wide applicability), it is also subject to a number of analytical and interpretive limitations (Unkovich et al., 2008). In addition, differences in the δ<sup>15</sup>N signal of the fixed-N and soil N are generally too small to allow for the fate of fixed-N to be tracked as it moves through the soil/plant continuum. Alternatively, <sup>15</sup>N-enrichment techniques—such as <sup>15</sup>N isotope dilution and <sup>15</sup>N<sub>2</sub> feeding—have been developed to quantify BNF (Knowles and Blackburn, 1993; Unkovich et al., 2008) and N rhizodeposition (Fustec et al., 2010; Leinweber et al., 2013).

Nitrogen-15 enrichment methods involve labelling either the soil (indirect) or the plant (direct) with <sup>15</sup>N. The <sup>15</sup>N isotope dilution technique involves (i) adding <sup>15</sup>N-labelled fertilizer (e.g., <sup>15</sup>N-

urea or  $^{15}\text{NH}_4^{15}\text{NO}_3$ ) to the soil in which an  $\text{N}_2$ -fixing (target) crop and a non- $\text{N}_2$ -fixing (reference) crop are grown; (ii) determining the amounts of total and  $^{15}\text{N}$  in the shoots of the target and reference crops at harvest; and (iii) calculating the percentage of N derived from the atmosphere (%Ndfa) (Hardarson and Danso, 1993; Unkovich et al., 2008; Fustec et al., 2010). Applying the  $^{15}\text{N}$ -label directly to the soil, however, makes it difficult, if not impossible, to track the fate of any fixed-N released during rhizodeposition or residue decomposition in the soil.

Supplying the  $^{15}\text{N}$  isotope label directly to the plant is more effective than labelling the soil (Wichern et al., 2008). Applying  $^{15}\text{N}$  to areal parts of the plant is a common approach—*shoot labelling* and *leaf immersion* being two examples of this technique (Janzen and Bruinsma, 1989; Mahieu et al., 2007). These techniques are relatively simple, do not require technically advanced equipment, and can be applied to a variety of crop species. One of the more commonly used shoot labelling methods is the *stem-wick method* (Wichern et al., 2008) in which a cotton wick immersed in  $^{15}\text{N}$ -urea (or  $^{15}\text{NH}_4\text{Cl}$  or  $\text{K}^{15}\text{NO}_3$ ) solution is threaded into the stem of the plant (Russell and Fillery, 1996; Arcand et al., 2013; Leinweber et al., 2013). Plant uptake of the  $^{15}\text{N}$ -labelled solution occurs via the transpiration stream. Although the stem-wick method is used widely—in part because it is easily maintained for long-term exposures—solution uptake efficiency is generally less than that of leaf-feeding techniques (Mahieu et al., 2007; Arcand et al., 2013). Leaf feeding methods involve  $^{15}\text{N}$ -labelling using a gas ( $^{15}\text{NH}_3$ ) (Janzen and Bruinsma, 1989) or a solution ( $^{15}\text{N}$ -urea or  $(^{15}\text{NH}_4)_2\text{SO}_4$  (Ledgard et al., 1985). The solution (leaf immersion) method is generally preferred because it is less technically demanding (Wichern et al., 2008); likewise, urea is the preferred carrier as it is non-polar, has a high N to mass ratio, and can be readily mobilized by urease. However, the quantity of  $^{15}\text{N}$  absorbed depends on the duration of exposure, and long-term exposures require technically advanced and expensive equipment that is difficult to adapt to field conditions (Bazot et al., 2008).

Most of the N taken up by plants enters the plant via the roots, thus root labelling methods have the advantage of more closely mimicking natural processes. The most common method of introducing a  $^{15}\text{N}$  label via the roots is the *split-root method* in which the plant root system is split between two compartments—only one of which is provided with  $^{15}\text{N}$  fertilizer. The roots in the non-labelled compartment are then used to determine  $^{15}\text{N}$ -enrichment of the roots and rhizodeposits. This method facilitates homogenous distribution of  $^{15}\text{N}$  through the plant and provide good estimates of N derived from rhizodeposition (Wichern et al., 2008).

Whereas NA methods can provide accurate estimates of BNF, they are not particularly useful for tracking the fate of this fixed-N in the soil/plant continuum or determining its contribution to the N nutrition of a subsequent, non-N<sub>2</sub> fixing crop. Conversely, leaf, stem and root labelling methods are well suited to tracking plant-derived N (i.e., N derived from rhizodeposition and residue decomposition), but provide no information regarding biologically fixed N. Thus, an approach is required that enables *in situ* labelling of the N fixed by a legume/pulse plant while at the same time allowing for subsequent recovery and quantification of the fixed-N in the above- and below-ground plant and soil compartments. One such method involves *atmospheric labelling*; i.e., adding <sup>15</sup>N<sub>2</sub> gas to the soil atmosphere (Leinweber et al., 2013).

Biological N fixation occurs by converting atmospheric N<sub>2</sub> into reactive N in the plant (see Section 2.1); thus labelling the soil atmosphere with <sup>15</sup>N<sub>2</sub> gas, the biologically fixed N stored in, and released by the plant retains some of the <sup>15</sup>N-label. This method has been used to evaluate N<sub>2</sub> fixation by legumes (Russelle et al., 1994; Russell et al., 1996) as well as non-symbiotic (associative) N<sub>2</sub> fixation (Bremer et al., 1995; Fustec et al., 2010). However, <sup>15</sup>N<sub>2</sub> atmospheric labelling is both technically complex and expensive (Wichern et al., 2008; Chalk et al., 2017), which has limited its adoption—mostly to controlled environment studies. Despite these drawbacks, and compared to other methods, direct labeling of legumes by exposing the roots and nodules to <sup>15</sup>N<sub>2</sub> gas provides the most accurate measure of N<sub>2</sub> fixation (Warembourg, 1993)—yielding a true representation of the contribution of fixed N to soil through N rhizodeposition and the decomposition of pulse crop residues.

### **3        QUANTIFICATION OF ABOVE AND BELOW GROUND NITROGEN FIXATION BY PULSE          CROPS USING DIRECT LABELLING OF THE SOIL ATMOSPHERE**

#### **3.1 Preface**

Nitrogen is an essential, yet one of the most limiting, plant nutrients in agro-ecosystems. Atmospheric di-nitrogen (N<sub>2</sub>) is the primary source of all reactive N forms found in plants and soil. Pulse crops, also known as grain legumes, have the ability to fix atmospheric N<sub>2</sub> through symbiotic association with *Rhizobium* bacteria, and a significant proportion of the N found in pulse crops is accumulated through biological nitrogen fixation. Thus, as a result of their ability to reduce the demand for externally applied N, suitability for the regional climatic conditions, and the growing global demand for the seeds as a protein source, pulse crops have become an integral part of western Canadian cropping systems. This study was carried out to quantify the above and below-ground N fixation of pulse crops important to the agro-economy of western Canada; i.e., pea, lentil, chickpea, and faba bean. The pulses were grown in specially designed rootbox system that exposed the plant roots to a <sup>15</sup>N<sub>2</sub>-enriched soil atmosphere. Biological nitrogen fixation by the pulses was determined by analyzing for the <sup>15</sup>N signal in the above- and below-ground plant components and calculating the amounts of N derived from the atmosphere (Ndfa). Nitrogen-15 enriched pulse residues and soil obtained at the end of this study will be used in a subsequent study to track the fate of the fixed N when wheat is grown in the residue amended soils.

### 3.2 Introduction

Cropping systems in the Canadian Prairies have become more diverse and environmentally sustainable (Bedard-Haughn et al., 2013) over the last few decades. Detrimental effects of fertilizer applications (Vitousek et al., 1997), challenging environmental conditions aggravated by climate change, and economic instability related to mono-cropping has motivated farmers to adapt alternative agricultural practices (Cutforth et al., 2007). As a result, most of the semi-arid regions in the Canadian Prairies have been changed from wheat-fallow systems to more diversified cropping systems (Miller et al., 2002). Indeed, the introduction of pulse crops has helped to reduce the use of summer fallow and increase cropping intensity (Larney et al., 1994; Li et al., 2011). Growing demand in the global market (Statistics Canada, 2016), the ability to perform under prairie climatic conditions (Cutforth et al., 2007) and the potential to reduce external N input cost due to N fixation (Walley et al., 2007) has made pulse crops an integral part of Canadian prairie cropping systems.

A pulse crop can fix 50 - 80% of its total N requirement through biological fixation. However most of the N fixed in a pulse crop is removed with the grain. In order to provide a positive N increment ( $N_{inc}$ ) to a subsequent crop, at least 48% of total plant N should come from biological N fixation (Walley et al., 2007). The nitrogen harvest index ( $\text{Grain-N} \div \text{Total Plant N}$ ) of pulses can vary from 0.5 to 0.9 (Walley et al., 2007; Hossain et al., 2017). Positive N increment (difference between the nitrogen derived from atmosphere (%Ndfa) and the nitrogen harvest index (NHI) (Walley et al., 2007) varies within different crops. For example, faba bean (*Vicia faba*) is recognized as a contributor to positive N increments while chickpea (*Cicer arietinum*) is unlikely to do so. Field pea (*Pisum sativum*) and lentil (*Lens culinaris*) are speculated to provide a positive N increment overtime (Walley et al., 2007). In addition to being able to provide a positive N increment, a pulse crop can provide an indirect N benefit, by sparing soil available N (Maidl et al., 1996). Mineralization is an important process that occurs continuously in soil. This enhances soil mineral-N pools (Stevenson and Van Kessel, 1996) by converting organic N from decomposing crop residues, animal manures, and labile soil organic matter to available N (Poth and Focht, 1985). Gan et al., (2010) estimated the net N mineralization under chickpea, dry pea and lentil by the time of maturity to be around 100 kg ha<sup>-1</sup>. Rhizodeposition is another mechanism that contributes to soil N pools (Wichern et al., 2008). It is speculated to be high during seedling to early flowering



stage due to the high nodulation, increased root exudates and possible sloughing off of nodules and root materials (Lupwayi and Soon, 2009). Pea plants have the ability to provide significant contribution of below ground N to total crop residue N. It was estimated that below ground N comprised 11.3% of total plant N for pea (Arcand et al., 2013) and 34% of total plant N for lentil at physiological maturity (Arcand et al., 2014). Fixed N in soil ecosystems transfers within soil and through the soil-plant system transforming into different reactive forms of N and ultimately gets released to the atmosphere (Rochette et al., 2006). Therefore, it is important to identify the N contribution of pulse crops to the soil and to subsequent crops when they are incorporated in crop rotations, in order to understand the dynamics of N flows for the entire cropping system. Insight into the N benefits of pulse crops and their residues (both above- and below-ground) should help producers manage fertilizer-N inputs of their cropping systems in a more efficient and effective manner.

Tracking the fate of fixed N through soil-plant systems has been attempted using a number of quantitative methods. Among the different isotopic and non-isotopic methods adapted to account for N-budgets in pulse crops, atmosphere labelling with  $^{15}\text{N}_2$  is the only direct approach that allows the distribution of fixed N through soil-plant system to be traced (Chalk., 2017). Even though the use of  $^{15}\text{N}_2$  to demonstrate legume–rhizobium symbiotic  $\text{N}_2$  fixation started more than 50 years ago (Burris et al., 1943), it was not widely used due to a number of reasons (Lesuffleur et al., 2013). Cost for  $^{15}\text{N}_2$  gas and mass spectrometer analysis was high compared to other widely used methods such as acetylene reduction assay. There were technical difficulties associated with designing laboratory set ups to run these experiments. For example, enclosing only the plant root was cost efficient, but providing leak-proof seal around the plant stem was a challenge. Environmental control (atmospheric concentrations of  $\text{CO}_2$ ,  $\text{O}_2$ , temperature, light intensity and humidity) within the chamber needed regular monitoring. In addition,  $^{15}\text{N}_2$  methods were only used for small scale single plant or nodule studies (Aprison et al., 1953; Leaf et al., 1958; Kennedy, 1966) whereas methods associated with the use of  $^{15}\text{N}$  fertilizer could be applied in large scale field studies (McNeil et al., 1994). Introduction of less expensive  $^{15}\text{N}_2$ , technological advancements in mass spectrometry (McNeil et al., 1994), and automated regulation of control environments (Chalk et al., 2017) attracted more researchers to use  $^{15}\text{N}_2$  techniques. Isotope labelling could be done either to the atmosphere of a growth chamber (Hopmans et al., 1983; McNeil et al., 1994; Ruschel et al., 1994; Carter and Ambus, 2006) or to the soil atmosphere

(Warembourg et al., 1982; Mohr et al., 1998; Lesuffleur et al., 2013; Van Phi Hung et al., 2013). However, most of these studies were done with forage legumes such as clover (Hopmans et al., 1983; Lesuffleur et al., 2013), and alfalfa (McNeil et al., 1994; Russelle et al., 1994; Mohr et al., 1998), or else for soybean (Warembourg et al., 1982; Van Phi Hung et al., 2013; Justino and Sodek; 2013). Apart from a few studies on common bean (Saito et al., 1980; Ruschel et al., 1994), and pea (Skøt, 1983, Sims et al., 1986), there have been very few studies using  $^{15}\text{N}_2$  labelling techniques to track fixed N for pulse crops.

The objective of this study was to grow pea, lentil, chickpea and faba bean simultaneously in a soil with continuous atmosphere labelling using  $^{15}\text{N}_2$  to quantify and compare biological nitrogen fixation by the four grain legumes. The contribution of fixed N to the soil N pool also will be determined.

### **3.3 Materials and Methods**

Biological  $\text{N}_2$  fixation by four pulse crops (pea, lentil, chickpea, and faba bean) was quantified using a  $^{15}\text{N}_2$  atmospheric labelling technique that involved growing the pulses—and a non-fixing reference plant (wheat)—in a *root box* system that included a  $^{15}\text{N}_2$ -enriched soil atmosphere. The root box system (RB- $^{15}\text{N}_2$ ) built for this study included design elements from an earlier closed loop  $\text{N}_2$  labelling system (Arcand, 2013), but was redesigned to reduce leakage and accommodate a larger number of plants. The RB- $^{15}\text{N}_2$  system was designed and constructed in-house by Frank Krijnen of the Department of Soil Science.

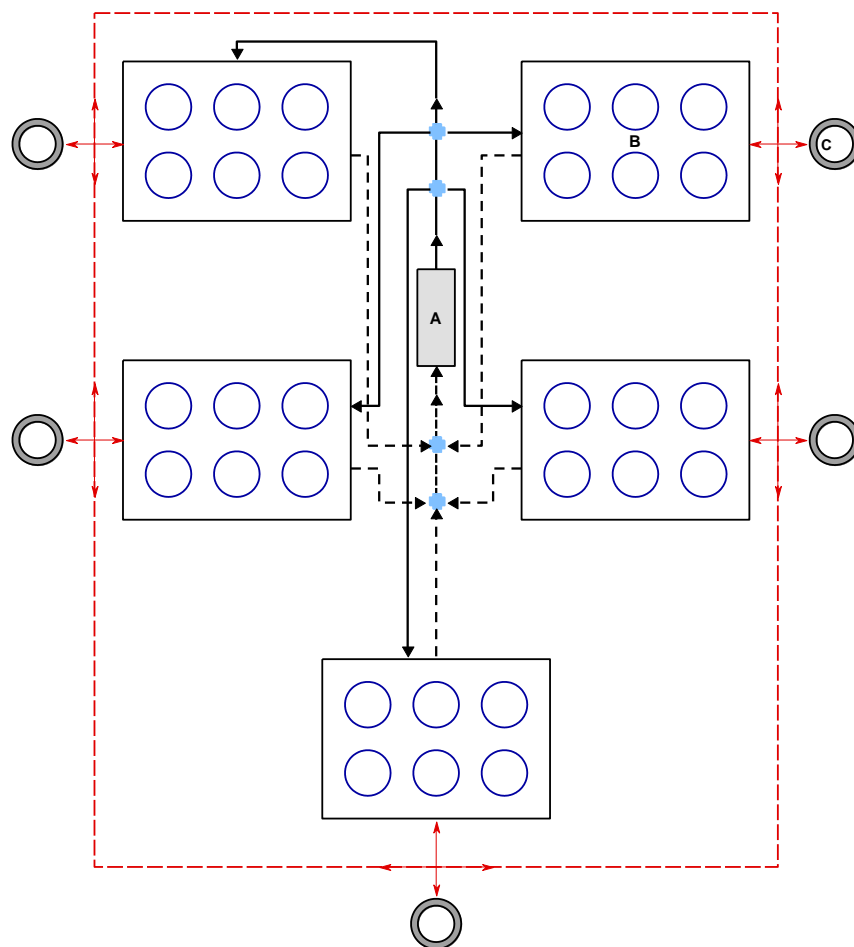
#### **3.3.1 Description of the RB- $^{15}\text{N}_2$ atmospheric labelling system**

The RB- $^{15}\text{N}_2$  system consisted of five interconnected root boxes (Fig. 3.1), each of which was comprised of six individual plant compartments. The individual plant compartments were constructed from PVC conduit (15-cm i.d.  $\times$  23.5-cm tall) sealed to a common rectangular PVC base (42 cm  $\times$  75 cm  $\times$  1.9 cm; Heritage plastics®, Harvey, IL) (Fig. 3.2), and were lined with a plastic bag (36 cm  $\times$  55.7 cm) before filling with a soil/sand mixture (see Section 3.4.2). Each root box was connected to an expansion bladder (i.e., a lawn mower tire tube [Kenda® 5.30/4.50-6; Kenda Tire Co., LTD., Yun Lin, Taiwan]) to prevent positive or negative pressures from developing in the root box in response to changes in atmospheric pressure. The expansion bladders were then inter-connected to prevent pressure build up within the system (see Fig. 3.1). All five

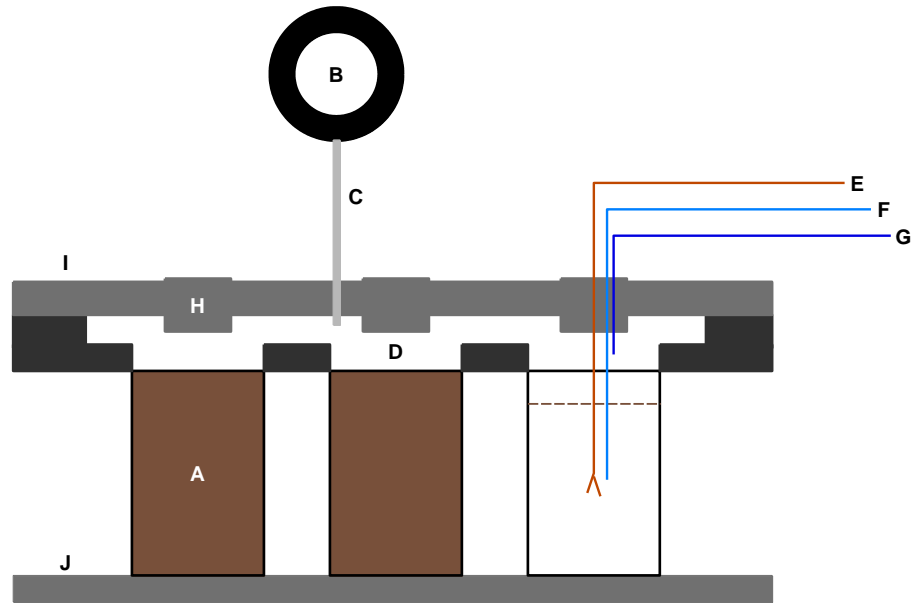
root boxes were connected to a central, closed gas circulating system consisting of a air pump (Parker Hargraves CTS micro diaphragm pump; Model # A.1C19N1.C06VDC; Hargraves Technology Corporation; Mooresville, NC) and 9.52 cm<sup>3</sup> mixing chamber (Fig. 3.1). The atmosphere in the RB-<sup>15</sup>N<sub>2</sub> system was mixed (2 L min<sup>-1</sup>) by operating the pump for 30 min every 12 h. Also included in each root box was a CO<sub>2</sub> trap consisting of a PVC column (6.27-cm i.d. × 23.9-cm) containing 800 g of soda lime that was located near the center of the box (see Fig. 3.1).

Each plant compartment included a soil moisture sensor (Decagon EC-5; Decagon Devices, Inc., Pullman, WA) inserted into the soil to a depth of 8.5 cm. In addition, inlet tubes for gas injection/sampling and watering (3.2-mm i.d.) were inserted into the soil to a depth of 8.5 cm. Once assembled, each root box was sealed with an acrylic lid (42 cm × 75 cm × 1.27 cm; Heritage plastics®, Harvey, IL) containing small (4.2-cm i.d.) holes that were centered over each plant compartment (Fig. 3.2). A short PVC tube (3.5-cm i.d. × 3-cm tall) was inserted through each hole—to act as a guide for the growing plant—and sealed into the lid using medical grade silicone (Silastic® MDX-4-4210; Dow Corning, Midland, MI). The lid was attached to the root box using stainless steel machine screws (#10-24), and was sealed in place using medical grade silicone (Silastic® MDX-4-4210, Dow Corning, Midland, MI). Silicone also was used to seal along the edges of the base and sides of the root box.

Once assembled, all five root boxes were connected as shown in Fig. 3.1, and placed under a metal tent (126 cm × 75 cm × 126 cm) consisting of two banks of T5HO fluorescent grow light bulbs (Grow Lights Canada, ON) bulbs providing a combination of blue (54 W, 6500K, 120 cm long) and red (54 W, 2700K, 120 cm long) light (Appendix, Fig. A1). Each light bank included 6 blue and 2 red lights. Light quality was controlled by changing the blue and red combinations of light to promote vegetative growth and reproductive growth. The light fixtures were attached to a moveable stand that allowed the lights to be raised as the plants matured, and were on a timer to provide a 16-h/8-h day/night photoperiod. The distance between the root box/plant apex and the light bank was maintained at *ca.* 30 cm to allow for air movement through the plant canopy and keep the temperature below 30°C.



**Figure 3.1. Schematic drawing of the RB- $^{15}\text{N}_2$  atmospheric labelling system showing the air flow through the headspace of root boxes. Arrows on the solid and dashed black lines show the direction of air flow entering (—) and exiting (---) the root boxes. Air was pumped through the gas mixing chamber (A) and into the root boxes (B), each of which was connected to an expansion bladder (C). The expansion bladders were then connected in series (---) to minimize pressure-induced changes in the air volume during operation of the system. Each root box included an air-permeable tube containing soda lime that acted as a  $\text{CO}_2$  trap.**



**Figure 3.2.** Side-view schematic of a root box showing three individual soil chambers (A). The expansion bladder (B) is connected via a valved gas line (C) to the shared headspace (D) of the root box. A soil moisture sensor (E) and gas inlet/sampling line (F), and water inlet (G) are inserted into the root box through an opening (H) in the lid (I). Note 1: each soil chamber is sealed to the base plate (J). Note 2: the soil moisture sensor and gas inlet/sampling line are inserted into the soil in each soil chamber; the water inlet is positioned above the soil surface in each chamber.

### 3.3.2 Preparation of soil medium and plants

Soil (0–15 cm; classified as an Orthic brown chernozem) was collected from a low-N field, under continuous wheat cultivation, at the U of S Goodale Research Farm (Saskatoon, SK) in November 2014. The soil was air dried, sieved (<4 mm) to remove rocks, and mixed 7:3 (v/v; 1:1 w:w) with silica sand (Granusil silica filler®; 94% silica sand, industrial grade: GHP Systems, Inc., Brookings, SD) to facilitate root recovery. The resulting soil/sand mixture had a loam texture, a pH of 7.3 (1:2 soil:water) and an EC of 0.2 mS cm<sup>-1</sup> (1:2 soil:water). Available nutrient concentrations in the soil/sand mix were: 9 µg NO<sub>3</sub>-N g<sup>-1</sup>, 29 µg P g<sup>-1</sup>, >300 µg K g<sup>-1</sup>, and 8 µg SO<sub>4</sub>-S g<sup>-1</sup>. Each individual plant compartment in the five root boxes was lined with a plastic bag and filled with 5.5 kg of the soil/sand mixture to yield a bulk density of 1.18 Mg m<sup>-3</sup>.

The pulse crops used in this study were field pea (*Pisum sativum*; var. CDC Treasure), lentil (*Lens culinaris*; var. CDC Viceroy), chickpea (*Cicer arietinum*; var. CDC Corinne), and faba bean (*Vicia faba*; var. FB 34-2; provided by Dr. R. Bueckert, Dept. of Plant Sciences, UofS). The reference

(non-fixing) crop was hard red spring wheat (*Triticum aestivum*; var. AC Barrie). For each crop, the plants were first germinated in Cone-tainers (3 seeds per Cone-tainer) filled with the same soil/sand mixture used in the root boxes. At the time of seeding, the pea, lentil and chickpea were inoculated using a peat-based commercial inoculant (i.e., TagTeam®: Novozymes BioAg, Saskatoon, SK) that was seed-applied according to the manufacturer's instructions, and at the recommended rates (i.e., equivalent to 1.63 g kg<sup>-1</sup> seed for pea and chickpea, and 2.67 g kg<sup>-1</sup> seed for lentil). Inoculant for the faba bean (*Rhizobium leguminosarum* bv. *viciae*; USDA 2507) was obtained from Dr. J. Diane Knight in the Dept. of Soil Science, and (using a solution containing 4 × 10<sup>8</sup> cfu mL<sup>-1</sup>) was applied at a rate of 1.25 mL per seed. Three weeks after germination, the seedlings (three per pot) were transplanted into each plant compartment. After one week the plants were thinned out leaving one plant per compartment. The water content of the soil in each compartment was initially adjusted to 60% field capacity (i.e., VSWC = 0.18 cm<sup>3</sup> cm<sup>-3</sup>). After transplanting, the root boxes were sealed as described in Sec. 3.3.1.

Whereas the <sup>15</sup>N-labelled pulse crops and non-fixing reference crop (wheat) were grown in the RB-<sup>15</sup>N<sub>2</sub> system, a companion set of non-labelled (i.e., natural abundance: NA) plants were grown in individual pots constructed from the same materials, and with the same dimensions, as the plant compartments in the root boxes. The NA reference plants (n = 6) were grown in a controlled environment chamber in the Phytotron (located in the College of Agriculture & Bioresources) in order to isolate them from the <sup>15</sup>N<sub>2</sub>-labelling system and ensure that there would be no cross-contamination from <sup>15</sup>N<sub>2</sub>. Likewise, a set of <sup>15</sup>N-labelled wheat plants were grown in the Phytotron by providing the plants with <sup>15</sup>N-labelled urea fertilizer (33 mg 10 atom% <sup>15</sup>N-urea). Environmental conditions (including light intensity, photoperiod and temperature) in the plant growth chamber were maintained as close as possible to those in the lab where the RB-<sup>15</sup>N<sub>2</sub> system was located (i.e., the AAFC Saskatoon Research Centre). Lighting in the phytotron was provided using 3500K high output bulbs (Philips F54T5/835/HO/EA/ALTO 49W, Philips Lighting Holding B.V, Eindhoven, the Netherlands).

### 3.3.3 <sup>15</sup>N<sub>2</sub> labelling and gas sampling

Prior to the start of <sup>15</sup>N<sub>2</sub> labelling, the integrity of each root box was assessed by performing a series of leak tests in which the root box (minus the plants) was sealed and a gas tracer (SF<sub>6</sub>)

injected into the soil compartments. The leak rate ( $L$ ) was then calculated from the SF<sub>6</sub> tracer data as described by Tingey et al. (2000); i.e., by monitoring the change in SF<sub>6</sub> concentration over time:

$$\frac{dC}{dt} = -LC_t \quad [\text{Eqn. 3.1}]$$

where  $L$  = leak rate, and  $C_t$  = the concentration of the SF<sub>6</sub> in the soil atmosphere (nL L<sup>-1</sup>) at time  $t$ . Integrating Eqn. 3.1 over time and solving for  $L$  gives:

$$L = \frac{\ln(C_0/C_t)}{t} \quad [\text{Eqn. 3.2}]$$

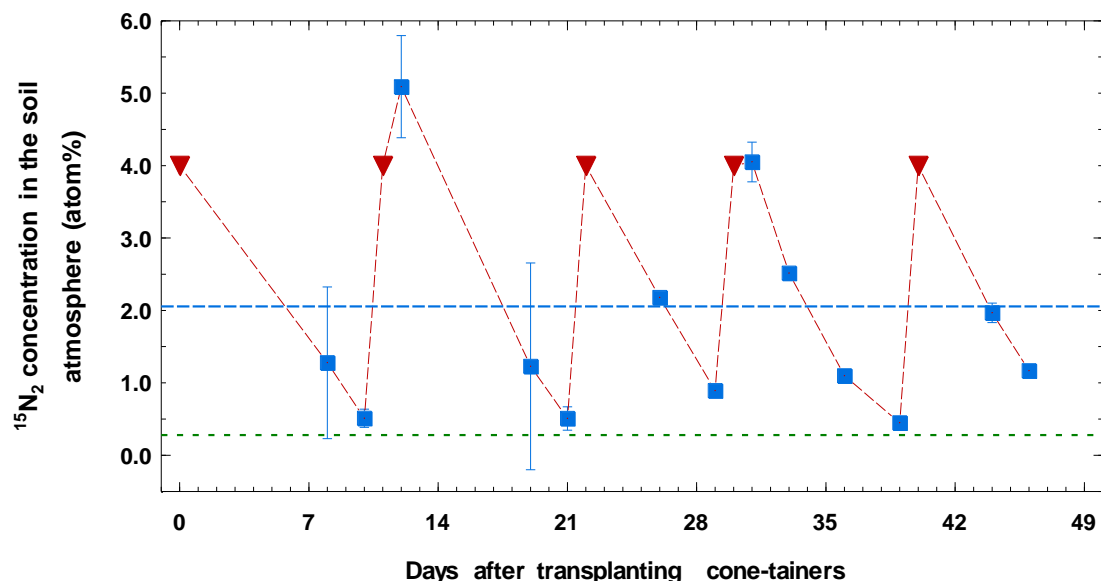
Initial tests revealed several large leaks in the root box system associated with faulty seals and tubing. Once located, all major leaks were repaired and the system retested. These preliminary tests with the RB-<sup>15</sup>N<sub>2</sub> system demonstrated that it was not possible to construct a completely “leak free” system, and that some <sup>15</sup>N<sub>2</sub> losses were inevitable. However, it was possible to minimize these leaks, such that the rate at which the tracer was lost from the root box system was relatively constant. Rearranging Eqn. 3.2 to solve for  $C_t$  yields:

$$C_t = C_0 e^{-Lt} \quad [\text{Eqn. 3.3}]$$

Using nonlinear regression techniques to fit Eqn. 3.3 to the SF<sub>6</sub> data, the  $L$  value averaged over five labelling cycles during the 46-d labelling period was determined to be -0.2123 d<sup>-1</sup> (± 0.0220 d<sup>-1</sup>;  $n = 5$ ;  $R^2 = 0.929 - 0.989$ ). Then, assuming that the ratio of <sup>15</sup>N<sub>2</sub> to SF<sub>6</sub> in the soil atmosphere remained constant (i.e.,  $L$  is the same for both SF<sub>6</sub> and <sup>15</sup>N<sub>2</sub>), the concentration of <sup>15</sup>N in the soil atmosphere at time =  $t$  was calculated as:

$$^{15}N_t = ^{15}N_{inj} \times e^{-0.2123t} \quad [\text{Eqn. 3.4}]$$

where  $^{15}N_t$  = atom% <sup>15</sup>N in the soil atmosphere at time  $t$ ; and  $^{15}N_{inj}$  = atom% <sup>15</sup>N in the soil atmosphere at the start of the labelling cycle. Applying Eqn. 3.4 over the 46-d labelling period yielded an average <sup>15</sup>N concentration of 2.0823 atom% (Fig. 3.3).



**Figure 3.3. Enrichment of  $^{15}\text{N}_2$  in the soil atmosphere of pulse crop labelling system.** Each pot in the six plant growth units was injected with 100 mL of  $^{15}\text{N}_2$  (98 atom%) along with 2.5 mL  $\text{SF}_6$  (1  $\mu\text{L/L}$ ). Concentrations of  $\text{SF}_6$  were determined by gas chromatography; leak rates were determined using Eqn. 3.4 applied to the data obtained during each labelling cycle. The average  $^{15}\text{N}_2$  concentration (2.0823 atom%) in the soil atmosphere is represented by the dashed blue line. Red triangles indicate the concentration in the soil atmosphere at the start of each labelling cycle. The dashed green line indicates the  $^{15}\text{N}_2$  concentration in the ambient atmosphere (i.e., 0.3663 atom%).

Seedlings grown in cone-tainers were transplanted into the root box system three weeks after sowing. For each plant species, three seedlings were transplanted into randomly selected soil compartments ( $n = 6$ ) in the root boxes (see Fig. 3.1) and allowed to become established (i.e., acclimatize to the new environment) for one week before the number of plants was reduced to one per soil compartment. Soil atmosphere labelling was initiated one week after the seedlings were transplanted into the root boxes<sup>1</sup>. The atmosphere in the root boxes was adjusted to contain  $3.9200 \pm 0.1960$  atom%  $^{15}\text{N}_2$  by injecting 0.10 L of 98 atom%  $^{15}\text{N}_2$  (Cambridge Isotope Laboratories Inc., Tewksbury, MA) into each soil compartment (see Fig. 3.1) for distribution throughout the RB- $^{15}\text{N}_2$  system. Prior to injection, an equivalent amount of air (0.6 L) was removed from the expansion bladders to ensure that the system would not become over-pressurized. The total amount of air in

<sup>1</sup> Note: it was observed that the transplanted seedlings had no prominent root nodules at the time they were transplanted into the root boxes. However, using a separate set of seedlings from all four pulse crops, it was determined that prominent nodules were present at 4-wk after sowing (i.e., 1-wk after transplanting)—at which time  $^{15}\text{N}_2$ -labelling of the soil atmosphere was initiated.



each root box (i.e., headspace + soil air-filled pore space in the plant compartments) was 15 L. The soil air-filled pore space was calculated as:

$$AFPS = \left(1 - \frac{VSWC}{TP}\right) \times 100 \quad [\text{Eqn. 3.5}]$$

where AFPS = air-filled pore space (%); VSWC = volumetric soil water content (%); and TP = total soil porosity (%), which was calculated from Eqn. 3.2:

$$TP = \left(1 - \frac{BD}{PD}\right) \times 100 \quad [\text{Eqn. 3.6}]$$

where BD = soil bulk density ( $1.18 \text{ g cm}^{-3}$ ) and PD = particle density ( $2.65 \text{ g cm}^{-3}$ ). As a result, the initial composition of root box atmosphere was 73%  $^{14}\text{N}_2$ , 4%  $^{15}\text{N}_2$  and 20%  $\text{O}_2$ ; the remaining 3% of the atmosphere consisted of water vapor, Ar and  $\text{CO}_2$ . In order to track the level of  $^{15}\text{N}_2$  in the root boxes, 13.8 mL of an  $\text{SF}_6$  standard ( $1 \text{ nL SF}_6 \text{ L}^{-1}$ ) was added as a gas tracer. Additional  $\text{SF}_6$  tracer was added each time the RB- $^{15}\text{N}_2$  system was supplemented with fresh  $^{15}\text{N}_2$  (i.e., 23  $\mu\text{L}$  of the  $\text{SF}_6$  standard per 1-mL of  $^{15}\text{N}_2$ ). To ensure that the gas sample was flushed into the soil, an additional 10 mL of lab air was slowly injected through the inlet tube after each  $^{15}\text{N}_2$  addition.

Soil moisture and oxygen composition were monitored daily during the first four weeks of the experiment and every second day thereafter. Soil moisture was monitored using the Decagon EC-5 soil moisture probes installed in each plant compartment. The composition of the soil atmosphere was monitored periodically by collecting a 20-mL sample from the headspace of each root box, transferring the sample into a pre-evacuated ( $6.58 \times 10^{-5} \text{ atm}$ ) 12-mL Exetainer® vial (Labco Ltd.; Lampeter, UK), and analyzing the sample for  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{SF}_6$  using a Bruker-Scion 456 gas chromatograph (Scion Instruments Canada, Mississauga, ON) equipped with a  $^{63}\text{Ni}$  electron capture detector and a thermal conductivity detector. In addition, real-time measurements of the  $\text{O}_2$  concentration in the soil atmosphere were made using a Qubit S-102  $\text{O}_2$  analyzer (Qubit Systems, Kingston, ON).

### 3.3.4 Plant harvest and analysis

All plants were harvested 10 weeks after transplanting; consequently, not all of the plants reached maturity or produced seed. Aboveground plant parts (stems, leaves, seeds and pods) were separated, dried at  $60^\circ\text{C}$  for one week, coarsely ground using a coffee grinder, and then finely

ground using a ball mill. The soil cores were destructively sampled and the roots recovered using tweezers. The soil was then passed through a 2-mm sieve to extract fragmented roots, that were then dried at 60°C. Sub-samples of the soils were dried at 105°C and finely ground using a ball mill. Sample weights were recorded before and after drying. Sub-samples (1–2 g, based on the amount of sample available) of all plant and soil samples were analyzed for total and  $^{15}\text{N}$  content using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc. CA) coupled to a Delta V mass spectrometer with ConFlo IV interface (Thermo Scientific in Bremen, Germany).

For both the  $^{15}\text{N}$ -labelled and NA samples, the bulk soils from the replicate plant compartments were combined (within each plant species) and homogenized using a cement mixer. The soils were then placed into pots (*ca.* 5 kg per pot), mixed with dried roots and stored at -20°C until needed for the Phase II study (see Chapter 4). A composite sample of the crop residue biomass (i.e., stems + leaves) for each crop species was also prepared, homogenized and stored at -20°C until needed for the Phase II study (see Chapter 4).

### 3.3.5 Nitrogen fixation calculations

Fixed nitrogen, i.e., nitrogen derived from the soil atmosphere (%Ndfa), was calculated using Eqn. 3.6 (Warembourg, 1993):

$$\%Ndfa = \frac{\text{atom\% } ^{15}\text{N excess plant}}{\text{atom\% } ^{15}\text{N excess soil atmosphere}} \times 100 \quad [\text{Eqn. 3.7}]$$

where  $^{15}\text{N}_2$  atom% excess in the soil atmosphere was determined by subtracting the NA value for atmospheric air (0.3663 atom%  $^{15}\text{N}$ ) from the mean atom%  $^{15}\text{N}$  value in the RB- $^{15}\text{N}_2$  system during the 46-d labelling period (i.e., 2.0823 atom%  $^{15}\text{N}$ ; calculated from the time-series data obtained following each addition of  $^{15}\text{N}_2$  to the root boxes). Biological nitrogen fixation (*BNF*) was then calculated using the %Ndfa and total N (*TN*) content of the plant (or plant component) as described in Eqn. 3.8 (Peoples et al., 2009):

$$BNF = \frac{\%Ndfa}{100} \times TN \quad [\text{Eqn. 3.8}]$$

Nitrogen derived from rhizodeposition (%NdfR) was calculated using Eqn. 3.9 (Janzen and Bruinsma, 1989):

$$\%NdfR = \frac{\text{atom}\% \text{ } ^{15}\text{N excess soil}}{\text{atom}\% \text{ } ^{15}\text{N excess roots}} \times 100 \quad [\text{Eqn. 3.9}]$$

where atom% excess values for the plant components and soil were obtained by subtracting the total atom%  $^{15}\text{N}$  in the reference (NA) soil and plant components from the total atom%  $^{15}\text{N}$  in the corresponding labelled samples.

### 3.3.6 Statistical Analyses

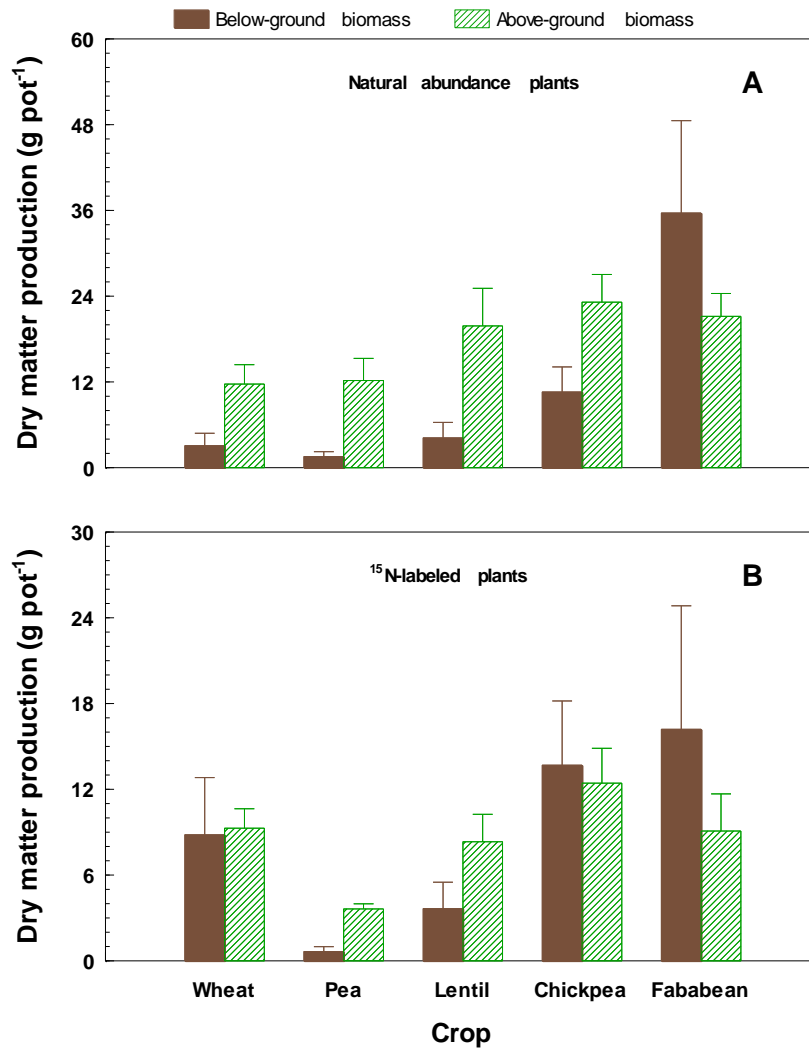
Treatments were allocated using a completely randomized design. Normality of the residuals were tested using D'Agostino-Pearson test and homogeneity of variance was tested using Levene's test. Means were compared using Tukey's Honest Significant test. All tests were declared significant if  $P \leq 0.05$ . All statistical analyses were performed using CoStat® statistics version 6.45 for Windows (Microsoft, 2008).

## 3.4 Results

### 3.4.1 Production of $^{15}\text{N}$ -labeled pulse crops

The ultimate purpose of the  $^{15}\text{N}_2$  enrichment method was to (i) provide a direct measure of BNF and (ii) produce plant residues with a  $^{15}\text{N}$  signature significantly different from that of the soil N pool—so that the fate of the fixed- $^{15}\text{N}$  could be tracked during the growth of a subsequent wheat crop. Plants were grown for 13 weeks under both natural abundance (NA) and  $^{15}\text{N}$ -enriched atmospheres; however, not all of the plants reached maturity and most were harvested at the late flowering to early pod filling stage. Plants grown in the RB- $^{15}\text{N}_2$  rootbox system generally produced less above-ground biomass than plants grown in the growth chamber (Fig. 3.4). However, below-ground (root) biomass production by plants grown in the rootbox system was generally comparable ( $p = 0.061$ ) to that of plants grown in the controlled environment chamber (Fig. 3.4)—the lone exception being faba bean. In both cases, however, there was a significant ( $P \leq 0.001$ ) crop type  $\times$  production method interaction; e.g., in the growth chamber, under natural abundance conditions, above-ground biomass production increased in the order: wheat  $\approx$  pea < faba bean  $\approx$  lentil  $\approx$  chickpea, whereas in the rootbox system it increased in the order: pea < lentil  $\approx$  wheat  $\approx$  faba bean < chickpea. Regardless of the production system, below-ground biomass production was greatest for faba bean and least for pea (Fig. 3.4). However, for plants grown in the growth chamber, under natural abundance conditions, below-ground biomass production

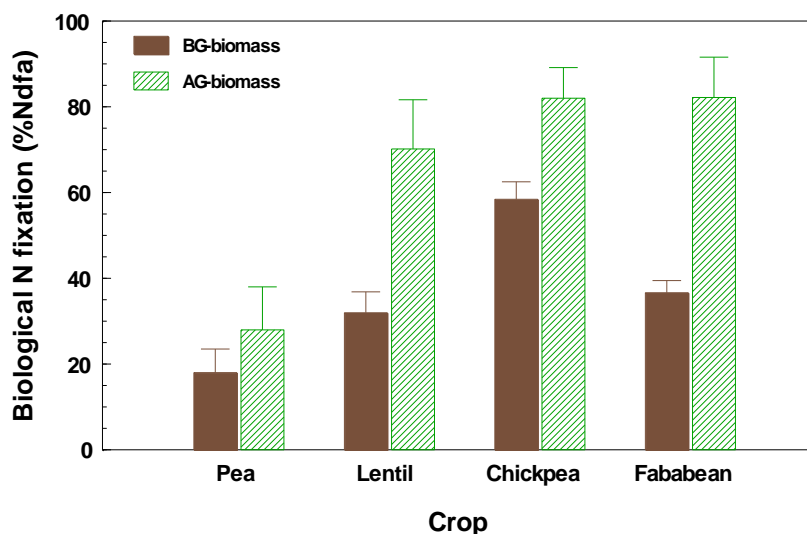
increased in the order: pea  $\approx$  wheat  $\approx$  lentil < chickpea < faba bean, whereas for the rootbox system it increased in the order: pea < lentil < wheat  $\approx$  chickpea  $\approx$  faba bean.



**Figure 3.4.** Total dry matter production of above-ground (stems+leaves+Pods) and below-ground (roots) residue of wheat, pea, lentil, faba bean and chickpea grown in a controlled environment chamber under ambient (natural abundance) conditions (A) or in the RB-<sup>15</sup>N<sub>2</sub> rootbox system under a <sup>15</sup>N<sub>2</sub>-enriched atmosphere (B). The rootbox system was pulse labeled with <sup>15</sup>N<sub>2</sub> gas (98 atom% <sup>15</sup>N<sub>2</sub>) throughout the growth period—yielding an average <sup>15</sup>N<sub>2</sub> concentration of 2.0823 atom% excess. Nitrogen-15 enrichment of the wheat was achieved using <sup>15</sup>N-labeled urea (33 mg 10-atom% <sup>15</sup>N) fertilizer.

### 3.4.2 Biological nitrogen fixation by pea, lentil, chickpea, and faba bean

Although plants grown in the rootbox system generally produced less above-ground biomass than plants grown in the growth chamber, this did not appear to adversely affect the ability of the plants to fix  $^{15}\text{N}_2$  from the soil atmosphere. Indeed, on a whole plant basis, the  $^{15}\text{N}$  enrichment of pulse crops grown in the rootbox system was 2.7- to 5.2-times greater than that of the pulse crops grown under ambient (natural abundance) conditions (i.e.,  $0.3665 \pm 0.0008$  atom%  $^{15}\text{N}$ ). In general, the amount of biologically fixed N in the above-ground biomass of the pulse crops was greatest for the faba bean and chickpea (82%Ndfa), intermediate for lentil (70%Ndfa), and least for pea (28%Ndfa). Conversely, the percentage of fixed N in the below-ground biomass (i.e., roots) was greatest for the chickpea (58%Ndfa), intermediate for the fababean (37%Ndfa) and lentil (32%Ndfa), and least for the pea (18%Ndfa) (Fig. 3.5).



**Figure 3.5.** Biological N fixation (%Ndfa) in above-ground residue (AG: stems+leaves+Pods) and below-ground residue (BG: roots) of pea, lentil, chickpea, and faba bean grown in the RB- $^{15}\text{N}_2$  rootbox system under a  $^{15}\text{N}_2$ -enriched atmosphere. The rootbox system was pulse labeled with  $^{15}\text{N}_2$  gas (98 atom%  $^{15}\text{N}_2$ ) throughout the growth period—yielding an average  $^{15}\text{N}_2$  concentration of 2.0823 atom% excess.

For a given pulse crop, the partitioning of  $^{15}\text{N}$  in the above-ground plant tissues (i.e., the stems, leaves, and pods) was relatively consistent (Table 3.1). In addition, with the exception of pea, differences among the pulse crops generally were not significant ( $p < 0.001$ ). Conversely,  $^{15}\text{N}$

enrichment of the below-ground plant parts (i.e., the roots) was species dependent—with the enrichment being greatest for chickpea. Indeed, for chickpea,  $^{15}\text{N}$  enrichment of the roots was comparable to that of the above-ground plant tissues, whereas for lentil and faba bean  $^{15}\text{N}$  enrichment in the roots was 30 to 38% lower than that of their above-ground plant parts. Likewise,  $^{15}\text{N}$  enrichment of the lentil and faba bean roots was 38 to 46% lower than that of the roots of chickpea.

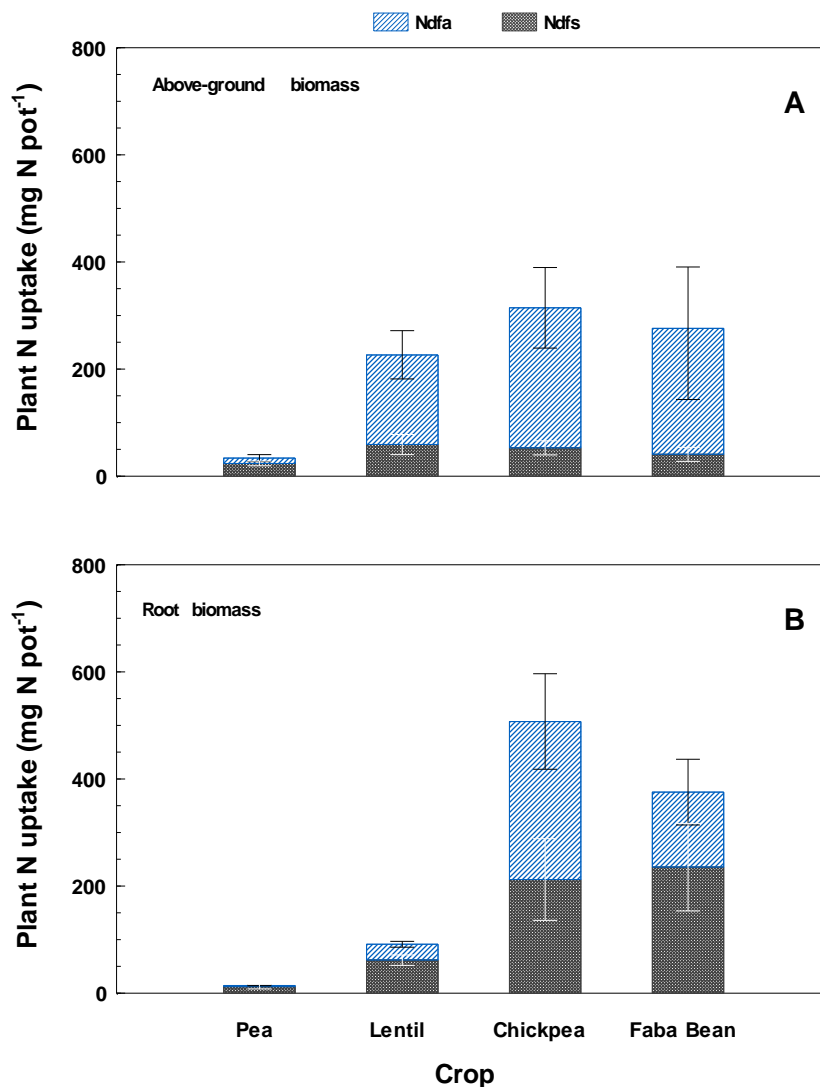
Whereas the data indicate that lentil, chickpea and faba bean allocated a larger percentage of their fixed N to the above-ground-biomass than to the below-ground biomass (Fig. 3.5), the lentil produced much smaller plants (with less root biomass) than either chickpea or faba bean (Table 3.2). Consequently, the total amount of fixed N in the roots was significantly ( $p < 0.001$ ) lower for the lentil (Fig. 3.6B). A similar trend was observed for the above-ground biomass (Fig. 3.6A), though differences in the amounts of fixed N in the lentil, faba bean and chickpea were much smaller and the treatment (crop species) means were not significantly different ( $p = 0.074$ ).

**Table 3.1. Nitrogen-15 enrichment ( $^{15}\text{N}$  atom% excess) in the above-ground plant components and roots of pulse crops grown in the RB- $^{15}\text{N}_2$  rootbox system with a  $^{15}\text{N}_2$  enriched soil atmosphere.**

Crop	Soil	Root <sup>†</sup>	Above-ground biomass			
			Stem	Leaves	Pods	Total <sup>‡</sup>
Pea	$0.0008 \pm 0.0004$ b	$0.4735 \pm 0.1446$ c	$0.3934 \pm 0.0984$ c	$0.4900 \pm 0.1956$ b	$0.5519 \pm 0.2687$ b	$0.6071 \pm 0.1814$ b
Lentil	$0.0052 \pm 0.0021$ a	$0.8413 \pm 0.1283$ b	$1.1740 \pm 0.1622$ b	$1.2106 \pm 0.2101$ a	$1.3523 \pm 0.2900$ a	$1.2612 \pm 0.1899$ a
Chickpea	$0.0074 \pm 0.0056$ a	$1.5470 \pm 0.1072$ a	$1.4236 \pm 0.1659$ a	$1.3910 \pm 0.1294$ a	$1.4432 \pm 0.1700$ a	$1.4127 \pm 0.1237$ a
Faba bean	$0.0088 \pm 0.0038$ a	$0.9645 \pm 0.0749$ b	$1.4175 \pm 0.1749$ a	$1.3407 \pm 0.2106$ a	$1.5192 \pm 0.1023$ a	$1.5523 \pm 0.1964$ a

<sup>†</sup> Within columns, means  $\pm$  standard deviations followed by the same letter are not significantly different ( $p < 0.05$ ; Tukey-Kramer test).

<sup>‡</sup> Values reported are the weighted averages for the combined above-ground biomass.



**Figure 3.6.** Total plant N in the above-ground (stems+leaves+Pods; A) and below-ground (roots; B) plant compartments of pea, lentil, chickpea, and faba bean grown in the RB-<sup>15</sup>N<sub>2</sub> rootbox system under a <sup>15</sup>N<sub>2</sub>-enriched atmosphere. The rootbox system was pulse labeled with <sup>15</sup>N<sub>2</sub> gas (98 atom% <sup>15</sup>N<sub>2</sub>) throughout the growth period—yielding an average <sup>15</sup>N<sub>2</sub> concentration of 2.0823 atom% excess. Nitrogen derived from the soil (Ndfs) was calculated as total N – nitrogen derived from the atmosphere (Ndfa).

Nitrogen-15 enrichments of the composite pulse crop residues (i.e., stems + leaves + pods + roots) were estimated (weighted average) to be 1.1007, 1.4910, and 1.1344 atom% excess for the lentil, chickpea, and faba bean, respectively. These values compared favorably with those obtained from IRMS analysis of the actual composite samples, which yielded <sup>15</sup>N enrichments of 1.1276, 1.6122, and 1.1620 atom% excess for the lentil, chickpea, and faba bean, respectively.



**Table 3.2. Above- and below-ground biomass production and nitrogen content of pulse crops grown in the RB-<sup>15</sup>N<sub>2</sub> rootbox system with a <sup>15</sup>N<sub>2</sub> enriched soil atmosphere.**

Crop	----- Root biomass -----				----- Above-ground biomass <sup>†</sup> -----			
	Yield (g pot <sup>-1</sup> )	N content (%)	TN (mg N pot <sup>-1</sup> )	BNF <sup>§</sup> (mg N pot <sup>-1</sup> )	Yield (g pot <sup>-1</sup> )	N content <sup>‡</sup> (%)	TN (mg N pot <sup>-1</sup> )	BNF <sup>§</sup> (mg N pot <sup>-1</sup> )
Pea	0.75 ± 0.25	2.12 ± 0.10	14.4 ± 4.5	2.25 ± 0.68	3.63 ± 0.63	1.05 ± 0.47	33.6 ± 8.8	9.92 ± 6.44
Lentil	4.05 ± 1.57	2.10 ± 0.39	91.2 ± 14.0	25.6 ± 5.4	8.33 ± 1.93	2.30 ± 0.44	227 ± 61	167 ± 45
Chickpea	13.69 ± 4.49	3.76 ± 0.67	507 ± 161	295 ± 89	12.43 ± 2.44	2.24 ± 0.12	314 ± 71	261 ± 75
Faba bean	13.45 ± 5.11	2.81 ± 0.24	375 ± 142	140 ± 61	9.08 ± 2.60	2.86 ± 0.53	267 ± 110	235 ± 123

<sup>†</sup> Above-ground biomass = stems + leaves + pods.

<sup>‡</sup> Values reported are the weighted averages for the combined above-ground biomass.

<sup>§</sup> Within columns, means ± standard deviations followed by the same letter are not significantly different ( $p < 0.05$ ; Tukey-Kramer test).

### 3.4.3 Nitrogen-15 uptake by wheat

Wheat biomass production was relatively unaffected by the  $^{15}\text{N}$ -labeling of the urea fertilizer (Table 3.3), but unlike the pulse crops, the wheat reached maturity during the 13-wk growth period. As a result, total N uptake was greatest in the seed—reflecting both its high yield and high N content. Wheat grown in soil amended with  $^{15}\text{N}$ -labeled urea exhibited significant ( $p < 0.001$ )  $^{15}\text{N}$  enrichment in both the above- and below-ground plant parts (Table 3.3). As was the case for the pulse crops,  $^{15}\text{N}$  enrichment of the above-ground plant parts was relatively uniform (ranging from 0.3104 to 0.3304 atom% excess) and, on average, was about 10% greater ( $p \leq 0.05$ ) than that of the roots.

**Table 3.3. Nitrogen content and  $^{15}\text{N}$  enrichment (atom% excess) in the above- and below-ground plant components of wheat fertilized with  $^{15}\text{N}$ -labeled urea or non-labeled urea.**

Plant part	N source <sup>†</sup>	Yield <sup>‡</sup> (g pot <sup>-1</sup> )	N content <sup>‡</sup> (%)	TN <sup>‡</sup> (mg N pot <sup>-1</sup> )	$^{15}\text{N}$ enrichment <sup>‡</sup> (atom% excess)
Seeds	NA-urea	5.00 ± 2.23 b	2.09 ± 0.64 a	95 ± 37 b	0.0011 ± 0.0005 b
	$^{15}\text{N}$ -urea	7.27 ± 1.23 a	1.98 ± 0.27 a	141 ± 14 a	0.3104 ± 0.0136 a
Stems	NA-urea	2.80 ± 1.03 a	0.55 ± 0.36 a	14 ± 6 a	0.0015 ± 0.0011 b
	$^{15}\text{N}$ -urea	2.19 ± 0.52 a	0.38 ± 0.13 a	8 ± 3 a	0.3198 ± 0.0094 a
Leaves	NA-urea	6.44 ± 1.42a	0.94 ± 0.41 a	78 ± 46 a	0.0012 ± 0.0009 b
	$^{15}\text{N}$ -urea	3.20 ± 0.56 b	0.74 ± 0.34 a	23 ± 10 b	0.3304 ± 0.0225 a
Chaff	NA-urea	3.42 ± 0.70 a	0.77 ± 0.41 a	22 ± a	0.0011 ± 0.0009 b
	$^{15}\text{N}$ -urea	3.89 ± 0.39 a	0.64 ± 0.16 a	25 ± 6 a	0.3146 ± 0.0149 a
Roots	NA-urea	3.85 ± 1.64 b	0.93 ± 0.20 a	37 ± 20 b	0.0006 ± 0.0007 b
	$^{15}\text{N}$ -urea	8.82 ± 4.00 a	1.05 ± 0.19 a	68 ± 25 a	0.2857 ± 0.0108 a

<sup>†</sup> Wheat plants were grown in the controlled environment chamber and received an application of non-labelled (natural abundance: NA) urea (0.1516 g urea-N pot<sup>-1</sup>) or  $^{15}\text{N}$ -labeled urea (0.0138 g urea-N pot<sup>-1</sup>).

<sup>‡</sup> Within columns, and for individual plant parts, means ± standard deviations followed by the same letter are not significantly different at the  $p = 0.05$  level of probability (Tukey-Kramer test).

The composite wheat residue (i.e., stems + leaves + chaff + roots) had an estimated  $^{15}\text{N}$  enrichment of 0.3058 atom% excess; IRMS analysis of the actual composite sample yielded a  $^{15}\text{N}$  enrichment of 0.3035 atom% excess.

### 3.5 Discussion

#### 3.5.1 Plant growth

Pulse crops grown in the labelling system developed successfully to produce sufficient shoot and root and biomass to be used as N inputs for a subsequent wheat crop. However, above-ground dry matter (straw) production of the pea plants was within the range reported by Yang et al., (2017); i.e., 1.8–4.7 g plant<sup>-1</sup> for straw and 0.27–0.73 g plant<sup>-1</sup> for roots. Arcand et al., (2013a) reported straw biomass for pea (3.76 g plant<sup>-1</sup>) that was comparable to what was obtained in the present study, though the root biomass (0.32 g plant<sup>-1</sup>) was less than half that obtained in the present study (0.65 g plant<sup>-1</sup>). These data suggest that pea grown in the rootbox system allocated more resources to root development—possibly because of poor nodulation. Indeed, it was observed during root recovery that there were few nodules present on the roots of the pea (Fig. 3.7), which was reflected in the relatively small amounts of <sup>15</sup>N<sub>2</sub> fixed by the pea (see Table 3.1). When symbiotic N fixation is inhibited, root growth is enhanced (Voisin et al., 2002) to increase the plant's ability to absorb nutrients (especially NO<sub>3</sub><sup>-</sup>) from soil. Thus, it is possible that the plants directed more resources to the roots in an effort to more effectively mine the available soil N pool. Regardless, overall development of the pea plants was poor (Fig. 3.7D)—as was both shoot and root biomass production—therefore, pea residues were not included in the follow-up study (Chapter 4).

In a study of the below-ground contribution of crop residues to the N nutrition of a subsequent wheat crop, Arcand et al. (2014) reported that at harvest (i.e., 70 days after seeding) 10% and 90% of lentil residue (i.e., total biomass - seed biomass) was in the roots and shoots, respectively. In the present study, 33% and 67% of the residue produced by lentil grown in the rootbox system was recovered in the roots and shoots, respectively. This compares favorably with the root-to-shoot ratio of field grown lentil at the flowering stage (0.34) reported by Gan et al. (2010). It should be noted that Arcand et al. (2014) grew the lentil to full maturity and reported that root dry matter production decreased during seed production as assimilates were translocated from the roots to the seeds. This presumably contributed to the lower root biomass in their study.



**Figure 3.7. Root systems of faba bean (A), chickpea (B), lentil (C) and pea (D) recovered from the RB-<sup>15</sup>N<sub>2</sub> root box system.**

Unlike lentil and pea, chickpea and faba bean produced much larger root systems (Fig. 3.7), with root-to shoot ratios of 1.1 and 1.5, respectively. For chickpea, the root-to-shoot ratio of plants grown in the rootbox system was similar to that reported by Khan (1998) for 10-wk old plants grown in a greenhouse (i.e.,  $1.02 \pm 0.20$ ) and by Ögütçü et al. (2009) for 7-wk old plants ( $0.89 \pm 0.20$ ). For faba bean, the root-to-shoot ratio of plants grown in the rootbox system was greater than that reported by Belachew & Stoddard (2017) for 11-wk old plants grown in a greenhouse (i.e.,  $0.33 \pm 0.15$ ).

### **3.5.2 Biological Nitrogen fixation**

The N in pulse crops comes from both the soil N pool and BNF. In this study, BNF—expressed as %Ndfa on a whole plant basis—increased in the order: pea < lentil  $\approx$  faba bean < chickpea. With the exception of pea, the Ndfa values for the pulse crops were comparable to those obtained with the same crops (and same varieties) grown in a companion field study at the University of Saskatchewan Goodale Research & Teaching Farm near Saskatoon (Farrell et al., 2017), as well as with those reported in several reviews of the literature (Unkovich and Pate, 2000; Walley et al., 2007; Peoples et al., 2009; Anglade et al., 2015; Hossain et al., 2016). On the other hand, the BNF by pea grown in the RB- $^{15}\text{N}_2$  rootbox system (35% Ndfa) was well below the mean and median values reported in the literature (Walley et al., 2007; Anglade et al., 2015; Hossain et al., 2016, Yang et al., 2017) or found in the companion field study (i.e., 55%). This was consistent with the overall poor performance (and poor nodulation) of pea in this study.

The partitioning of Ndfa throughout the pulse crops varied with plant species (see Tables 3.1 and 3.2). For example, in terms of atom% excess and total fixed N, lentil and faba bean had more fixed N in the above-ground plant compartments than in the roots, whereas the fixed N in chickpea was distributed more evenly throughout the plant. In general, N fixation peaks between flowering and pod filling, after which it decreases (Kurdali, 1996; Viosin et al., 2002; Fischinger and Schulze, 2010; Arcand, 2013). This decrease in N fixation occurs as a result of the onset of nodule senescence, competition for C between the nodules and reproductive organs, and decreased growth rate of plants. As well, fixed N is translocated into the seeds as the plants reach physiological maturity (Kurdali et al., 1997; Schiltz, et al., 2005). Indeed, Arcand et al. (2013b) reported that most of the fixed N in field pea at maturity was detected in the seeds, whereas the leaves contained greater amounts of fixed N at the vegetative and flowering stages. In the present study, the pulses

were harvested at the late flowering to early pod filling stage, with only a few plants—mainly faba bean—reaching maturity. As a result, most of the fixed N remained in the above-ground residue. Indeed, 63% of fixed N (42% of total N%) was detected in above-ground components of faba bean at the time of harvest. Between the early pod fill and maturity more than 80% of the N contained in above-ground components can be redistributed to the seeds (Dekhuijzen and Verkerke, 1984). As well, a greater percentage of the N detected in seeds at the maturity is derived from BNF (Westermann et al., 1985). Similar patterns have been observed in chickpea (Kurdali, 1996) and lentil (Kurdali et al., 1997).

When BNF and soil N uptake is not sufficient to meet the N demand during seed development, N is translocated from the vegetative parts of the plants to the pods. Research has shown that net mobilization of N from the roots and shoots to the pods ranges from 43–94% in lentil (Kurdali et al., 1997), and can be as high as 81% in chickpea (Kurdali, 1996). In the present study, the plants were only just starting to set seed at the time of harvest, and only 7–28% of total fixed N was recovered in the seeds. Consequently, the amounts of fixed N in the plant residues was likely greater than what could be expected had the plants been grown to physiological maturity.

The total amount of fixed N in the plant residues depends on both the %Ndfa and biomass production. For example, although root biomass production for chickpea (13.69 mg pot<sup>-1</sup>) was essentially the same as that for faba bean (13.45 mg pot<sup>-1</sup>), the total amount of fixed N in the roots of chickpea was about 37% greater than that in the roots of faba bean—reflecting the much greater <sup>15</sup>N enrichment of the chickpea roots. On the other hand, the roots of lentil and faba bean did not differ significantly in their <sup>15</sup>N enrichment, but faba bean produced about 3.3-times as much root biomass as lentil—resulting in about 4.4-times as much fixed N (375 vs. 91 mg pot<sup>-1</sup>) in the faba bean. Overall, my data support the findings of Khan et al. (2003) and Arcand et al. (2013a, 2014) that the below-ground N contribution of grain legumes has been greatly underestimated—if not ignored completely—and, hence, undervalued.

### **3.5.3 Plant nitrogen distribution**

Similar to biomass distribution, plant N content varies with plant development (Schiltz et al., 2005, Arcand et al., 2014). For example, in pea grown in the greenhouse (Arcand et al., 2013b) and the field (Gan et al., 2010), the above-ground N content of the plant increased during vegetative and flowering stages. In the study by Arcand et al. (2013b), the above-ground N content then decreased

by approximately 90% when the plants reached maturity, while at the same time, the root N content decreased by about only 61%. Gan et al. (2010) reported that the average root N content of field grown pea decreased linearly as the plants matured—with a similar trend being observed for lentil and chickpea. Khan et al., (2003) reported that the N recovered in the roots of chickpea and faba bean plants harvested just prior to maturity accounted for 77% and 25%, respectively, of total N. However, for plants grown in a greenhouse and harvested at maturity, total N recovery in the roots of chickpea and faba bean was only 53% and 39%, respectively (Khan et al., 2002). In the present study, approximately 53% and 37% of fixed N was recovered in the roots of chickpea and faba bean (see Table 3.2). Whereas, in part, this presumably reflects the fact that the plants did not reach physiological maturity—i.e., they were harvested prior to any significant remobilization of N from the roots to the pods/seeds. However, as the ultimate goal of this research was to track the fate of fixed N during residue decomposition, this was considered to be advantageous in that it provided residues with a stronger  $^{15}\text{N}$  signal that could be traced through soil-plant system.

#### **3.5.4 Nitrogen in the soil**

In addition to the fixed N in the roots themselves, rhizodeposition (i.e., the release of compounds containing fixed N) represents a potentially important source of mineralizable N. Indeed, studies have shown that rhizodeposition can account for as little as 4% and much as 71% of total fixed N (Mayer et al., 2003; Fustec et al., 2010; Arcand et al. 2013). To date, however, most studies investigating N rhizodeposition by grain legumes have involved field pea (Sawatsky and Soper, 1991; Jensen, 1996; Schmidtke, 2005; Arcand et al., 2013a), which unfortunately did not perform well in the current study. Moreover, these studies employed  $^{15}\text{N}$  labelling methods such the split-root labelling, stem-wick labelling, and  $^{15}\text{N}$ -isotope dilution that did not rely on BNF and, hence, may not accurately represent the processes involved in the mobilization and release of symbiotically fixed N.

In the present study, the only way for  $^{15}\text{N}$  to enter the soil was through fixation and release from the plant roots, the amount of fixed N released through rhizodeposition was established by determining the concentration (atom% excess) of  $^{15}\text{N}$  in the bulk soil at the time of harvest. In general, rhizodeposited  $^{15}\text{N}$  increased in the order: pea  $\ll$  lentil  $\approx$  chickpea  $\approx$  faba bean, with  $^{15}\text{N}$  enrichments ranging from 0.0008 to 0.0088 atom% excess. Based on these enrichment factors, it was calculated that less than 1% of total fixed N was released into the soil as rhizodeposits.

### 3.6 Conclusion

Stable isotope labelling—using  $^{15}\text{N}_2$  as the source of the label—provides a means of directly determining the amounts of atmospheric  $\text{N}_2$  taken up by pulse crops and other legumes through BNF, tracking the partitioning of fixed N through the plant system, and estimating the contribution of N rhizodeposition to the soil N pool. This study employed a rootbox system (RB- $^{15}\text{N}_2$ ) that introduced  $^{15}\text{N}$ -enriched  $\text{N}_2$  directly into the soil atmosphere to produce  $^{15}\text{N}$ -enriched residues of pea, lentil, chickpea, and faba bean that could then be used to track the fate of the fixed N during a subsequent growth study with wheat.

All four pulse crops fixed significant amounts of  $\text{N}_2$  from the atmosphere, with Ndfa accounting for 35–86% of total N uptake on a whole-plant basis. With the exception of pea, which exhibited poor nodulation, the pulse crops exhibited BNF values that were within the ranges reported in the literature. Nitrogen-15 enrichment of the roots indicated that Ndfa accounted for 18–58% of the total root N and 13–53% of total Ndfa—indicating the potential importance of the below-ground N to soil N fertility. The high  $^{15}\text{N}$  enrichments of the pulse residues (both above- and below-ground residues) and relatively low enrichment of the soils in which they were grown, will make it possible to track the fate of the fixed  $^{15}\text{N}$  when wheat is grown in the residue amended soils. Due to its overall poor performance, however, pea residue will not be used in the follow-up study.



## **4      QUANTIFICATION OF THE NITROGEN CREDIT PROVIDED BY CROP RESIDUES, AND IDENTIFICATION OF SOIL EMITTED NITROUS OXIDE SOURCES DURING THE WHEAT PHASE OF A PULSE-CEREAL ROTATION**

### **4.1 Preface**

The inclusion of pulses (or grain legumes) in crop rotations provides a number of nitrogen and non-nitrogen benefits. Fixed nitrogen in pulse crop residues enrich the soil N pools and can increase the yield of subsequent crops. In addition, their inclusion in rotations can restrict root and shoot diseases, reduce weed growth, increase nutrient (other than N) and organic matter content, help sequester carbon. The incorporation of pulse crop residues into the soil can reduce the amount of fertilizer-N needed by the subsequent crop, which may reduce emissions of soil-derived nitrous oxide (N<sub>2</sub>O)—a major greenhouse gas (GHG) emitted from agricultural systems. However, some of the N in the pulse residues will be converted into N<sub>2</sub>O in the soil, thus offsetting—at least partially—the reductions achieved by decreasing fertilizer-N inputs. This study was carried out to track the fate of biologically fixed N in pulse residues during the growth of a subsequent wheat crop. Wheat was grown in soils amended with <sup>15</sup>N-enriched pulse residues and analyzed (plant matter and soil) for residue-derived <sup>15</sup>N. In addition, GHG samples were collected throughout the growth period to quantify the cumulative N<sub>2</sub>O emissions and determine the contribution of the pulse residue to these emissions. The <sup>15</sup>N-enriched pulse residues were obtained from the study described in Chapter 3, in which four pulse crops; pea, lentil, chickpea, and faba bean were grown in a soil atmosphere labelled with <sup>15</sup>N<sub>2</sub>. As the pea crop performed poorly and did not produce sufficient biomass, pea residue was not included as a N input in this study.

## 4.2 Introduction

Cropping systems in the Canadian prairies have changed from cereal-fallow systems to more diverse and environmentally sustainable cropping systems over the past few decades (Bedard-Haughn et al., 2013) and integration of pulse crops has played a key role in making this shift (Hossain et al., 2016). Pulse crops provide rich plant proteins for human and animal consumption, and act as a renewable source of N for agricultural soils (Peoples et al., 2009). Due to their ability to fix atmospheric N, pulses have a low demand for externally added fertilizer (Beckie and Brandt, 1997) and can be grown in nutrient poor soils (Adderley et al., 2006). In addition, fixed N is less susceptible to loss through volatilization, denitrification and leaching than is fertilizer-N (Jensen and Hauggaard-Nielsen, 2003). Not surprisingly, therefore, the emission of N-based greenhouse gases is also reported to be lower in systems that include pulses compared to cereal or cereal-oilseed based systems (Lemke et al., 2007). This, in turn, reduces the carbon footprint of the grain products in a pulse-cereal cropping system (Gan et al., 2014).

Inclusion of grain legumes in crop rotations increases the yield of subsequent cereal crops (Wright, 1990; Rowland et al., 1994; Steven and van Kessel, 1996; Beckie et al., 1997; Beckie and Brandt, 1997; Gan et al., 2001; Soon and Clayton, 2002; Soon and Arshad, 2004; Miller et al., 2003; Gan et al., 2003; Jensen et al., 2009; Chen et al., 2012; St. Luce et al., 2015; Lupwayi and Soon, 2016). In addition to grain yield, Lupwayi and Soon (2016) reported that N uptake, C accumulation, and straw dry matter were greater in wheat grown on legume residues compared to wheat grown on barley residues. However, the magnitude of the N benefit depends on the quality of the residue (St. Luce et al., 2015), crop type (both legume and subsequent crop) (Lupwayi and Soon, 2016), and site-specific conditions such as soil texture, organic C content, acidity, the supply of available soil-N, and the preceding crop; as well as on environmental conditions such as precipitation and air and soil temperature (St. Luce et al., 2015). The nitrogen fixing potential of pulse crops varies with crop type; but with proper inoculation, pulses are reported to obtain 50% to 80% of their total N through biological N fixation (Walley et al., 2007).

The “N benefit” (i.e., N increment [ $N_{inc}$ ]) provided by a pulse crop to a subsequent crop depends on how much fixed N is retained in the crop after the grain has been removed (harvested). Thus, there is a positive  $N_{inc}$  only when the amount of fixed-N in the pulse residues exceeds the amount exported with the grain (Toomsan et al., 1995; Crozat and Fustec, 2006; Walley et al., 2007). In a

4-yr rotational study involving pulse crops (used as green manure and grown for seed), a forage legume, cereals, and an oilseed crop, Lupwayi and Soon (2015) observed cereal crop yields increased when preceded by a pulse crop, but there was no significant yield response for oilseed crops preceded by a pulse crop. They estimated that 63% of the N in faba bean residues and 50% of the N in pea residues was released during the first year. However, this value was much greater when the pulses were grown as a green manure than when they were grown for seed. Similar observations were made by Chen et al. (2012) and St. Luce et al. (2015), who reported that grain protein content and total N uptake were greater when preceded by a forage or grain legume green manure.

Inclusion of a pulse in rotation with a cereal also has been shown to enhance the long-term contribution of crop residues to soil N pools, and improve the N supplying power of the soil, for a wheat-lentil cropping sequence compared to a continuous wheat sequence (Zentner et al., 1998; Campbell et al., 2005). Moreover, it has been observed that the yield increase in a subsequent crop often exceeds the level expected solely from the N received from the pulse crop residues. Thus a portion of the yield increase has been attributed to non-N benefits (Stevenson and van Kessel, 1996; Beckie and Brandt, 1997; St. Luce et al., 2015; Lupwayi and Soon, 2016).

The C:N ratio of crop residues determines whether N is mineralized or immobilized when added to soil. In general, immobilization and N sequestration dominates when residues with a high C:N ratio are added (Delgado et al., 2010), whereas mineralization dominates when residues with a low C:N ratio are added (Baggs et al., 2000; Gupta, 2016). Pulse crop residues have a low C:N ratio compared to cereal residues. Thus, rotations with pulse crops provide readily decomposable C and nutrients for respiration and the growth of micro-organisms—promoting mineralization in soil (Gupta, 2016). There is still considerable uncertainty regarding the N contribution of different pulse crop residues to subsequently grown crops, and more research accurately tracing the quantity and fate of the fixed-N in pulse crop residues is needed. More reliable estimates of the relative contribution of N from pulse residues would help producers manage N inputs to their cropping systems with greater efficiency and effectiveness.

In Canada, the agriculture sector accounts for 8% (59 Mt) of total greenhouse gas (GHG) emissions, with livestock and fertilizer-N being the main drivers of these GHG emissions (Environment Canada, 2017). Indeed, in 2015, 42% of total agricultural emissions came from

livestock digestion (i.e., enteric fermentation), while application of inorganic N fertilizers accounted for 22% of total agricultural emissions (Environment Canada, 2017). In the case of N fertilizers, emissions of N<sub>2</sub>O from managed soils occur either directly from the soils to which the N is added (or from N released through mineralization) or indirectly through (i) volatilization, fossil fuel combustion and biomass burning; or (ii) leaching and runoff (IPCC, 2006). Thus, to reduce GHG emissions associated with crop production, it has been suggested that one need only reduce external N inputs to cropping systems (Gan et al., 2015). One way of doing so, is to include pulse crops in rotation with cereal and oilseed crops (Lemke et al., 2007; Jeuffroy et al., 2013). However, the effect of replacing mineral N fertilizer with biological N fixers has been much debated among researchers (Peyrard et al., 2016). Nitrogen in crop residues (including forage and grain legume residues) is considered a source of direct N<sub>2</sub>O emissions from cropping systems (IPCC, 2006). Indeed, most of the N<sub>2</sub>O emissions associated with pulse crops can be attributed to residue decomposition after harvest (Rochette and Janzen, 2005), rather than during biological N fixation by the pulse crops (Zhong et al., 2009). Residues with low C:N ratios have been shown to enhance N<sub>2</sub>O emissions as they provide readily available substrates for nitrification and denitrification (Baggs et al., 2000; Huang et al., 2004). However, in cropping systems with high organic matter accumulation and C sequestration, the N in crop residues is less susceptible to loss via N<sub>2</sub>O emission or NO<sub>3</sub><sup>-</sup> leaching compared to the N in synthetic fertilizers (Delgado and Follett, 2002).

Greenhouse gas emissions are often expressed in terms of an emission factor. For N<sub>2</sub>O, this is the amount of N<sub>2</sub>O-N emitted per unit amount of added N (IPCC, 2006). According to the current IPCC methodology for direct soil-emitted N<sub>2</sub>O, mineral fertilizers, organic amendments and crop residues, and N mineralized from mineral soils are considered to have a similar influence on N<sub>2</sub>O emissions and all are assigned an emission factor of 0.01 kg N<sub>2</sub>O-N (kg N)<sup>-1</sup> (with an uncertainty range of 0.003 to 0.03 kg N<sub>2</sub>O-N (kg N)<sup>-1</sup>) (Walsh and Williams, 2006). Although it is well known that the nitrogen derived from crop residues contributes to N<sub>2</sub>O emitted from cropping systems (Delgado et al., 2010), a number of researchers have argued that (i) crop residues and synthetic N fertilizer should not be considered to have the same emission factor for N<sub>2</sub>O (Lemke et al., 2002; Rochette et al., 2004; Rochette and Janzen, 2005; Helgason et al., 2005; Zhong et al., 2009; Lupwayi and Kennedy, 2007); and (ii) the IPCC methodology should be changed by lowering the N<sub>2</sub>O-N emission factor for crop residue-N (Dusenbury et al., 2008; Delgado et al., 2010; Barton

et al., 2011). However, accurate measurement of the N<sub>2</sub>O-N losses from crop residues requires the use of <sup>15</sup>N-labeled substrates, yet only a few such studies have been reported in the literature (Delgado et al., 2010). In particular, there is an almost complete lack of published data examining the contribution of biologically fixed nitrogen to the production and emission of N<sub>2</sub>O from soils—indeed, a search of the literature found only one such study, which examined biologically fixed N<sub>2</sub> as a source of N<sub>2</sub>O production in a grass-clover mixture (Carter and Ambus, 2006).

The objectives of this study were; (i) to quantify the supply of fixed N from pulse crops using <sup>15</sup>N labelled crop residues of chickpea, lentil, faba bean and wheat to the nitrogen uptake of the subsequent wheat crop and (ii) to assess N<sub>2</sub>O emission from soils amended with pulse and wheat crop residues to identify the sources of soil emitted N<sub>2</sub>O.

### **4.3 Materials and Methods**

The nitrogen benefits of pulse crop residues to a subsequent cereal (wheat) crop were evaluated using <sup>15</sup>N-enriched residues (both above- and below-ground residues) of three pulse crops (chickpea, lentil and faba bean) grown in a soil atmosphere labelled with <sup>15</sup>N<sub>2</sub>, and a reference crop (wheat) grown in soil amended with <sup>15</sup>N-enriched urea fertilizer. Total and residue-derived N<sub>2</sub>O were determined by taking gas samples from the headspace of the pots in which the wheat was grown. At harvest, the wheat plants and soil were analyzed for their total N and <sup>15</sup>N content.

#### **4.3.1 Preparation of <sup>15</sup>N-enriched pulse residues**

This study was conducted in a controlled environment using <sup>15</sup>N labelled crop residues of three pulse crops and wheat. The pulse crops were grown using a rootbox system (RB-<sup>15</sup>N<sub>2</sub>) that introduced <sup>15</sup>N-enriched N<sub>2</sub> directly into the soil atmosphere (see Chapter 3, Sec. 3.3.1) while the wheat plants were grown in the same soil, but were fed with <sup>15</sup>N-urea fertilizer (Chapter 3, Sec. 3.3.2). All plants were harvested 10 weeks after germination during the late flowering to early pod filling stages. Plant and soil samples were dried, weighed and analysed for both total N and <sup>15</sup>N content to determine the amount of biologically fixed N in the plant residues.

The labelling phase of the study also included a parallel set of non-<sup>15</sup>N enriched reference plants that were grown (and harvested) at the same time to provide background (natural abundance) measurements needed for subsequent calculation of BNF (Chapter 3, Sec. 3.3.2). The natural

abundance (NA) pulse crops were grown under the same conditions—with the exception that they were grown in a different location to ensure that there was no cross-contamination.

For both the  $^{15}\text{N}$ -enriched and NA treatments, soil from the replicate pots for each crop species was bulked and homogenized by mixing in a small cement mixer. Likewise, composite samples of the above- and below-ground plant residues were prepared for each crop species. The composite soil and root samples for each species were then combined, homogenized by mixing in a small cement mixer, and divided into replicate ( $n = 5$ ) pots; the above-ground residue was then divided into five sub-samples (total mass was dependent on crop species; see Table 4.1) and placed on top of the potted soil. The composite samples (soil + below-ground residue, and surface applied above-ground residue) were then frozen ( $-20^{\circ}\text{C}$ ) until needed for the wheat phase of the study.

**Table 4.1. Treatment (residue + fertilizer) combinations used in the wheat phase of the study.**

Trt. ID <sup>a</sup>	----- AGR <sup>b</sup> -----					----- BGR <sup>b</sup> -----					N fertilizer <sup>c</sup>		Total N	Total <sup>15</sup> N
	(g pot <sup>-1</sup> )	(%N)	(atom% <sup>15</sup> N)	mg N pot <sup>-1</sup>	mg <sup>15</sup> N pot <sup>-1</sup>	(g pot <sup>-1</sup> )	(%N)	(atom% <sup>15</sup> N)	mg N pot <sup>-1</sup>	mg <sup>15</sup> N pot <sup>-1</sup>	(mg N pot <sup>-1</sup> )	(mg <sup>15</sup> N pot <sup>-1</sup> )	mg N pot <sup>-1</sup>	mg <sup>15</sup> N pot <sup>-1</sup>
<sup>15</sup> N-b	--	--	--			--	--	--			--	--		
<sup>15</sup> N-W	9.4	0.67	0.6953	63.0	0.44	6.2	0.93	0.6528	57.7	0.38	67.20	0.25	187.84	1.06
<sup>15</sup> N-L	10.9	2.16	1.6231	235.4	3.82	1.6	1.91	1.3484	30.6	0.41	67.20	0.25	333.20	4.48
<sup>15</sup> N-C	17.5	2.54	1.8627	444.5	8.28	5.9	3.86	2.0566	227.7	4.68	67.20	0.25	739.44	13.21
<sup>15</sup> N-F	21.2	2.29	1.7096	485.5	8.30	4.3	2.69	1.3746	115.7	1.59	67.20	0.25	668.35	10.14
NA-b	--	--	--			--	--	--			--	--	--	--
NA-W	9.4	0.87	0.3676	81.8	0.30	6.2	0.93	0.3669	57.7	0.21	--	--	206.64	0.51
NA-W+ <sup>15</sup> NU	9.4	0.87	0.3676	81.8	0.30	6.2	0.93	0.3669	57.7	0.21	67.20	0.77 <sup>d</sup>	139.44	1.28
NA-L	10.9	2.24	0.3664	244.2	0.90	1.6	1.94	0.3672	31.0	0.11	67.20	0.25	342.40	1.26
NA-C	17.5	2.00	0.3658	350.0	1.28	5.9	2.28	0.3685	134.5	0.50	67.20	0.25	551.72	2.03
NA-F	21.2	2.27	0.3661	481.2	1.76	4.3	2.25	0.3669	96.8	0.36	67.20	0.25	645.19	2.37

<sup>a</sup> <sup>15</sup>N = <sup>15</sup>N-enriched; NA = natural abundance; b = blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean; U = urea.

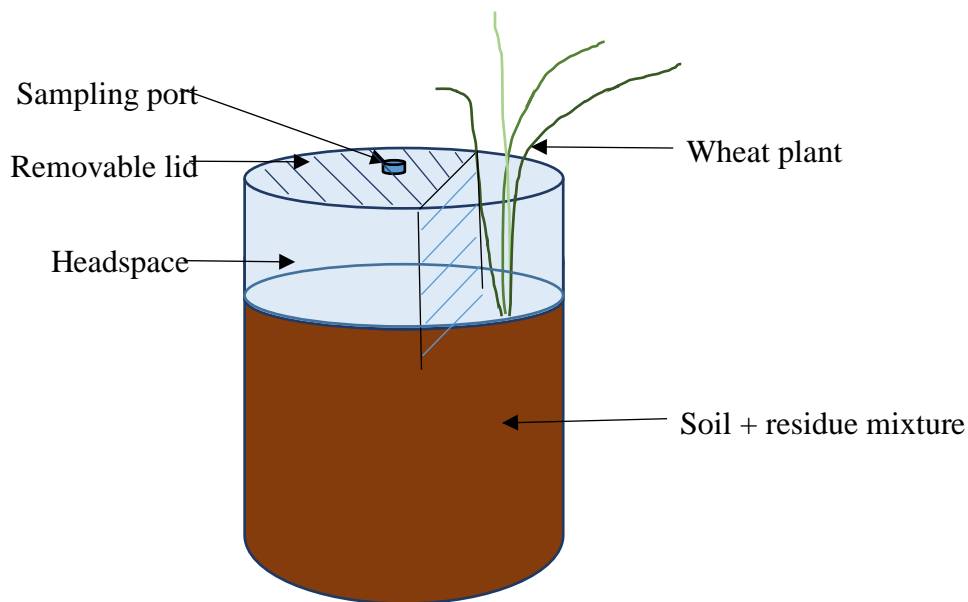
<sup>b</sup> BGR = below-ground residues (roots + rhizodeposits); AGR = above-ground residues (stems + leaves + pods).

<sup>c</sup> Nitrogen added as natural abundance (0.3663 atom% <sup>15</sup>N) urea (144 mg NA-urea per pot); all pots also received additions of K and P fertilizer at rates based on soil test results.

<sup>d</sup> Nitrogen added as <sup>15</sup>N-urea (i.e., 16.4 mg of 10 atom% <sup>15</sup>N-urea per pot); all pots also received additions of K and P fertilizer at rates based on soil test results.

#### 4.3.2 Establishment and maintenance of the wheat phase

At the start of the wheat phase of the study, the frozen soils with the residues were slowly thawed to room temperature, at which time the above-ground residues were mixed into the upper (0–5 cm) layer of the soil. Headspace partitions (13.5 cm × 5.0 cm) were fixed to the pots using silicon glue (Silastic® MDX-4-4210, Dow Corning, Midland, Michigan) (Fig. 4.1). Lids for the pots were prepared with a gas sampling port and grooves along the underside of the lid that fit the perimeter of the pot. (Note: when in place for gas sampling, the lids were sealed to the pots using vacuum grease [Dow Corning® High vacuum grease, Midland, Michigan] applied to the grooves in the lid.)



**Figure 4.1.** Schematic of the pot designed to obtain gas samples from wheat growing soils amended with pulse crop residues. Headspace of the pot was divided into two compartments using a PVC partition (F). One was used to grow the wheat plant (D) and the other dedicated to collect soil emitting gas (C). Compartment C had a lid (B) with a septum (A) to insert the needle of the sampling syringe.

The soils were watered to 60% field capacity and weighed. The soil water content was maintained at  $55 \pm 5\%$  field capacity by reweighing the pots every second day and adding the required amount of water (i.e., weight @ 60% FC – daily weight = weight of water to be added). Wheat (AC Barrie) was seeded directly into the residue-amended soil (6–8 seeds per pot) as shown in Fig. 4.1. After germination, the wheat was thinned to a final plant density of four plants per pot.



All treatments except the blanks and labelled urea + non-labelled wheat residue received 160.4 mg of urea (equivalent of 40 kg ha<sup>-1</sup>). Labelled treatments received the same amount of urea as a combination of <sup>14</sup>N and <sup>15</sup>N labelled urea (16.4 mg of 10 atom% excess <sup>15</sup>N urea + 144 mg of NA urea). All pots received P (0.028 g pot<sup>-1</sup>; equivalent to 35 kg ha<sup>-1</sup>) and K (0.075 g pot<sup>-1</sup>; equivalent to 50 kg ha<sup>-1</sup>).

#### 4.3.3 Gas sampling

Greenhouse gas (N<sub>2</sub>O and CO<sub>2</sub>) sampling was initiated during the thaw period, continued for 10 weeks, and was terminated two weeks prior to harvest. The wheat was grown in specially designed pots that allowed the gas samples to be collected without disturbing the plants (see Figs. 4.1 and 4.2). Gas sampling was performed daily during the first week, once every 2 days during the second week, and once a week for the remainder of the growth period. On each sampling date, the pot was covered without disturbing the plants (see Fig. 4.2) for 30 minutes and three sets of gas samples were collected from each pot. The first set of gas samples (*ca.* 20 mL) was injected into pre-evacuated 12-mL Exetainer® vials (LabCo Inc.; High Wycombe, UK), while the second set of samples (*ca.* 30 mL) was injected into pre-evacuated 22-mL screw-top glass culture tubes (Kimble™ Kimax; Thermo Fisher Scientific, Ottawa, ON) fitted with butyl rubber septa and analyzed at the *Prairie Environmental Agronomy Research Laboratory* (PEARL) in the Department of Soil Science at the University of Saskatchewan in Saskatoon. [Note: the third set of samples—also injected into pre-evacuated 22-mL screw-top glass culture tubes—were kept as a backup in case there was a problem with one of the other samples.] Gas samples stored in the 12-mL Exetainer® vials were analyzed for total N<sub>2</sub>O concentration using gas chromatography (SCION 456GC equipped with a <sup>63</sup>Ni electron capture detector). Gas samples in the 22-mL glass culture tubes were analysed for <sup>15</sup>N<sub>2</sub>O concentration using cavity ring-down spectroscopy (CRDS; Picarro G5301-*i* isotopic N<sub>2</sub>O analyzer). Daily N<sub>2</sub>O fluxes (mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup>) were plotted versus elapsed time (d) and cumulative emissions were calculated using an area-under-the-curve (AUC) analysis. The total cumulative N<sub>2</sub>O-N and cumulative <sup>15</sup>N<sub>2</sub>O-N emission data were then used to calculate emission factors (EF) for the different crop residues.



**Figure 4.2. Pots with the gas sampling lids in place. The left-hand panel also shows the syringe (20-cc) and needle (1", 22-gauge) used to sample the chamber headspace.**

#### **4.3.4 Plant and soil analysis**

The wheat was grown until it reached maturity and was harvested 13 weeks after seeding. At harvest, the wheat plants from each pot were collected; separated into seeds and stems + leaves; placed in a forced air oven and dried at 60°C for one week, and then weighed. The pots were destructively sampled with the root material and soil collected separately. The plant materials (stems + leaves, roots) were coarsely ground using a coffee grinder, and sub-sampled—with the subsamples being finely ground using a ball mill. To avoid contamination, the  $^{15}\text{N}$ -enriched samples were processed separately from the natural abundance samples—using equipment reserved for isotopically enriched samples. The soils were also sub-sampled and finely ground using the ball mills. The finely ground soil and plant samples were analyzed using isotope ratio mass spectrometry (IRMS)—with a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., CA) interfaced to a Thermo Delta V mass spectrometer with a ConFlo IV interface (Thermo Scientific in Bremen, GDR)—to determine total-N and atom%  $^{15}\text{N}$ . These values were then used to quantify the amount of fixed  $^{15}\text{N}$  taken up by the wheat and the amount remaining in the soil.

Total inorganic N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) was determined by extracting the soil with 2.0 M KCl using a 1:2 soil:extractant ratio (Maynard et al., 2008) followed by colorimetric analysis using a Technicon Autoanalyzer (Technicon Industrial Systems, Tarrytown, NY). Total N was determined (Figueiredo, 2008) by dry combustion using a LECO TruMac CNS analyzer (LECO Corp., Saint Joseph, MI).

#### 4.3.5 Calculations

Fixed nitrogen in pulse crops, i.e., nitrogen derived from the atmosphere (%Nd<sub>fa</sub>), was calculated using Eqn. 3.3 (Warembourg, 1993) and nitrogen derived from rhizodeposition (%Nd<sub>fR</sub>) was calculated using Eqn. 3.4 (Janzen and Bruinsma, 1989) (Section 3.4.5).

Nitrogen contribution of residues to the subsequent crop, i.e., nitrogen derived from the residues (%Nd<sub>fr</sub>), was calculated using Eqn. 4.1 (Hauck and Bremner, 1976):

$$\%Nd_{fr} = \frac{\text{atom\% } ^{15}\text{N excess in wheat (subsequent crop)}}{\text{atom\% } ^{15}\text{N excess in residue}} \quad [\text{Eqn. 4.1}]$$

#### 4.3.6 Statistical Analyses

Treatments of this study were allocated as a completely randomized design. Normality of the residuals were tested using D'Agostino-Pearson test and homogeneity of variance was tested using Levene's test. Unless otherwise noted, all means comparisons were made using the Least Significant Difference (LSD) test. All tests were declared significant at  $P = 0.05$ . Analysis was done using CoStat® statistics version 6.45 for Windows (Microsoft, 2008).

### 4.4 Results

#### 4.4.1 Seed yield and biomass production

Wheat was grown in soils amended with <sup>15</sup>N-enriched pulse crop residues to trace the fate of the residue N in terms of both the N nutrition of the wheat and the contribution of the pulse residues to N<sub>2</sub>O emissions from the soil. The soils were amended with <sup>15</sup>N-labelled plant residues (above- and below-ground residues) of lentil, chickpea, faba bean, and wheat grown in the RB-<sup>15</sup>N<sub>2</sub> rootbox system under a <sup>15</sup>N<sub>2</sub>-enriched atmosphere (see Chapter 3 for details). The wheat was grown to maturity and harvested 13 weeks after seeding.

In addition to the wheat grown in soil amended with the  $^{15}\text{N}$ -labelled residues, wheat also was grown in soil amended with non-labelled (i.e., natural abundance) residues that were produced in parallel with the  $^{15}\text{N}$ -labelled residues (see Chapter 3 for details). These plants were grown solely to provide a natural abundance  $^{15}\text{N}$  reference for each residue type, and an analysis of variance revealed that in terms of yield there were no significant ( $P = 0.296\text{--}0.786$ ) differences between wheat grown in soil amended with the  $^{15}\text{N}$ -labelled residues and wheat grown in soils amended with the NA residues. Thus, only the results obtained for the wheat grown in soils amended with the  $^{15}\text{N}$ -enriched residues will be presented and discussed in this chapter.

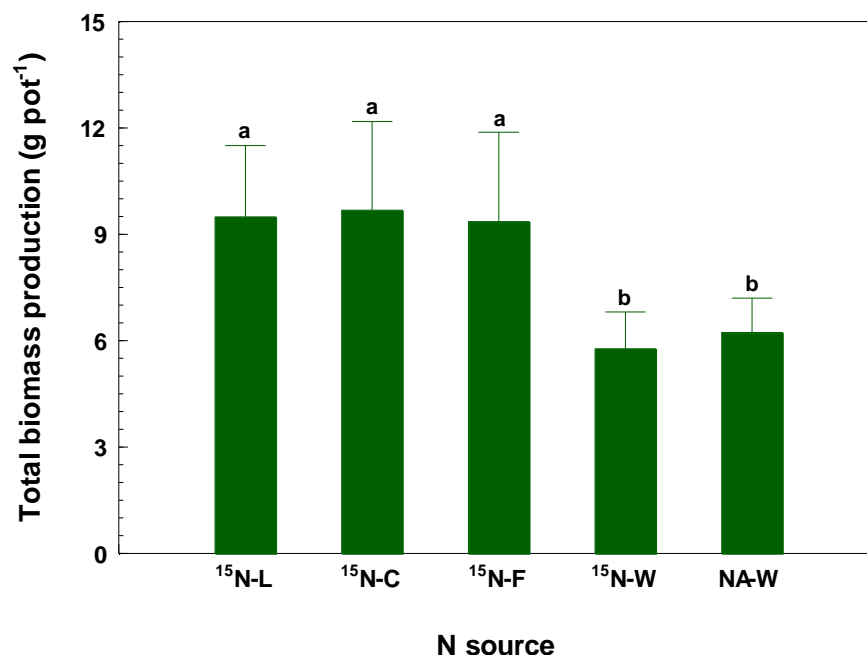
In general, wheat grown in the soils amended with pulse crop residues produced greater ( $P < 0.001$ ) seed yields ( $4.22 \pm 1.01 \text{ g pot}^{-1}$ ) than the wheat grown in soils amended with wheat residues ( $2.75 \pm 0.49 \text{ g pot}^{-1}$ ). Likewise, total biomass production (i.e., straw + root + seed) was greater for wheat grown in the pulse residue-amended soils than in the wheat residue-amended soils (Fig. 4.3). Despite these differences, however, there were no significant differences ( $P = 0.272$ ) in the harvest index (HI; Table 4.2). At the same time, however, root-to-shoot ratios were generally greater ( $P = 0.050$ ) for wheat grown in soils amended with pulse residues than for wheat grown in soils amended with wheat residues (Table 4.2). The type of pulse residue had no significant effect on either seed yield or biomass production (Table 4.2 and Fig. 4.3).

**Table 4.2. Seed yield, root and straw biomass production of wheat plants.**

Treatment ID <sup>†</sup>	Root biomass ----- g pot <sup>-1</sup> -----	Straw biomass ----- g pot <sup>-1</sup> -----	Seed yield ----- g pot <sup>-1</sup> -----	Root:Shoot ratio	Harvest Index
$^{15}\text{N-L}$	$0.93 \pm 0.34 \text{ a}$	$4.44 \pm 0.94 \text{ a}$	$4.12 \pm 0.87 \text{ a}$	$0.21 \pm 0.05 \text{ a}$	$0.48 \pm 0.02 \text{ a}$
$^{15}\text{N-C}$	$0.88 \pm 0.47 \text{ a}$	$4.60 \pm 1.13 \text{ a}$	$4.21 \pm 0.94 \text{ a}$	$0.18 \pm 0.06 \text{ abc}$	$0.48 \pm 0.02 \text{ a}$
$^{15}\text{N-F}$	$0.82 \pm 0.26 \text{ a}$	$4.21 \pm 1.08 \text{ a}$	$4.33 \pm 1.21 \text{ a}$	$0.19 \pm 0.02 \text{ ab}$	$0.51 \pm 0.02 \text{ a}$
$^{15}\text{N-W}$	$0.43 \pm 0.10 \text{ b}$	$2.75 \pm 0.34 \text{ b}$	$2.59 \pm 0.69 \text{ b}$	$0.15 \pm 0.02 \text{ bc}$	$0.49 \pm 0.06 \text{ a}$
NA-W	$0.40 \pm 0.07 \text{ b}$	$2.91 \pm 0.65 \text{ b}$	$2.91 \pm 0.29 \text{ b}$	$0.14 \pm 0.03 \text{ c}$	$0.50 \pm 0.03 \text{ a}$

<sup>†</sup>  $^{15}\text{N}$  =  $^{15}\text{N}$ -enriched; NA = natural abundance; blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean.

<sup>‡</sup> Within columns, means  $\pm$  standard deviations followed by the same letter are not significantly different ( $P = 0.05$ ; LSD test).



**Figure 4.3.** Total biomass production (i.e., roots + shoots + seed) by wheat grown in soils amended with <sup>15</sup>N-labelled residues of lentil (<sup>15</sup>N-L), chickpea (<sup>15</sup>N-C), faba bean (<sup>15</sup>N-F), and wheat (<sup>15</sup>N-W); or with natural abundance (NA) wheat residue supplemented with <sup>15</sup>N-labelled urea fertilizer (NA-W). Bars labeled with the same letter are not significantly different at the  $P = 0.05$  level of probability (LSD test).

#### 4.4.2 Recovery of residue-derived nitrogen by wheat

Wheat plants grown in soil amended with <sup>15</sup>N-enriched residues of lentil, chickpea, faba bean, and wheat were significantly ( $P < 0.001$ ) enriched with <sup>15</sup>N (Table 4.3). Moreover, total recovery of the residue-derived <sup>15</sup>N by the wheat plants was greatest by plants grown in soils amended with pulse crop residues (Fig. 4.4). In general, there was a strong positive correlation between the amount of <sup>15</sup>N recovered in the wheat plants and the amount of <sup>15</sup>N added to the soil as residue (i.e.,  $r = 0.978$ ;  $P < 0.004$ ), with the amount of <sup>15</sup>N recovered increasing in the order: wheat residue  $\ll$  lentil residue  $<$  faba bean residue  $\leq$  chickpea residue. In terms of percent recovery, however, wheat grown in soil amended with lentil residues recovered a greater percentage of the residue N (13.6%) than wheat grown in soils amended with either the chickpea (9.2%) or faba bean (10.9%) residues (Table 4.4). Plants grown in soil amended with non-labelled (natural abundance) wheat residues plus a small amount of <sup>15</sup>N-urea (i.e., equivalent to *ca.* 5 kg N ha<sup>-1</sup>) recovered about 3-times more <sup>15</sup>N than plants grown in soil amended with <sup>15</sup>N-enriched wheat residues (Fig. 4.4 and Table 4.4), though the difference was not significant.

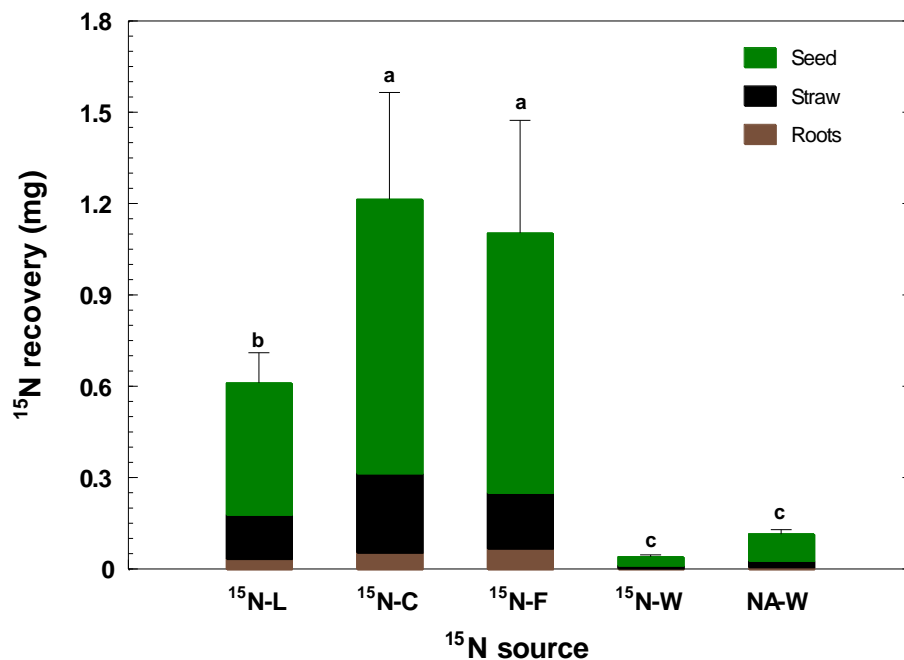
**Table 4.3. Nitrogen-15 concentration in the above- and below-ground plant compartments of wheat grown in soils amended with <sup>15</sup>N-enriched pulse crop or wheat residues.**

	Roots	Straw <sup>§</sup>	Seed
<sup>15</sup> N source <sup>†</sup>	----- <sup>15</sup> N (atom% excess) <sup>‡</sup> -----		
<sup>15</sup> N-L	0.3656 ± 0.0206 b	0.3479 ± 0.0230 b	0.3629 ± 0.0183 b
<sup>15</sup> N-C	0.6291 ± 0.0663 a	0.5981 ± 0.0514 a	0.6552 ± 0.0551 a
<sup>15</sup> N-F	0.7034 ± 0.0761 a	0.6195 ± 0.0485 a	0.6796 ± 0.0487 a
<sup>15</sup> N-W	0.0465 ± 0.0092 c	0.0467 ± 0.0086 c	0.0449 ± 0.0022 c
NA-W/ <sup>15</sup> N-U	0.1817 ± 0.0303 c	0.1487 ± 0.0404 c	0.1324 ± 0.0228 c

<sup>†</sup> <sup>15</sup>N = <sup>15</sup>N-enriched; NA = natural abundance; blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean.

<sup>‡</sup> Within columns, means ± standard deviations followed by the same letter are not significantly different (LSD; *P* < 0.05).

<sup>§</sup> Values reported are the weighted averages for the stems + leaves.



**Figure 4.4. Nitrogen recovery in the roots, straw and seeds of wheat plants grown in soils amended with <sup>15</sup>N-labelled pulse crop and wheat residues. A soil amended with non-labelled (natural abundance) wheat residue supplemented with <sup>15</sup>N-labelled urea fertilizer (equivalent to ca. 5 kg <sup>15</sup>N ha<sup>-1</sup>) was included as a reference treatment. Bars labelled with the same letter are not significantly different (LSD; *P* = 0.05).**

Residue-derived  $^{15}\text{N}$  was greatest (on both a total amount and percent recovery basis) in the wheat seeds and least in the wheat roots (Table 4.4). However, only about 4% to 14% of total residue  $^{15}\text{N}$  was recovered in the wheat plants themselves, with the majority of the remaining residue-derived  $^{15}\text{N}$  being recovered in the soil—including N recovered as non-decomposed residue (data not shown).

**Table 4.4. Nitrogen-15 recovery in the above- and below-ground plant compartments of wheat grown in soils amended with  $^{15}\text{N}$ -enriched pulse crop or wheat residues.**

$^{15}\text{N}$ source <sup>†</sup>	Roots	Straw <sup>§</sup>	Seed	Total
	----- mg $^{15}\text{N}$ <sup>‡</sup> -----			
$^{15}\text{N}$ -L	0.033 ± 0.009 bc	0.145 ± 0.016 b	0.433 ± 0.080 b	0.611 ± 0.099 b
$^{15}\text{N}$ -C	0.054 ± 0.026 ab	0.260 ± 0.070 a	0.900 ± 0.258 a	1.214 ± 0.351 a
$^{15}\text{N}$ -F	0.067 ± 0.021 a	0.183 ± 0.058 b	0.853 ± 0.306 a	1.103 ± 0.370 a
$^{15}\text{N}$ -W	0.002 ± 0.001 c	0.009 ± 0.004 c	0.029 ± 0.005 c	0.040 ± 0.006 c
NA-W/ $^{15}\text{N}$ -U	0.006 ± 0.002 c	0.021 ± 0.007 c	0.088 ± 0.011 c	0.114 ± 0.015 c
	----- % recovery <sup>¶</sup> -----			
$^{15}\text{N}$ -L	0.73 ± 0.20 a	3.24 ± 0.37 a	9.64 ± 1.79 a	13.64 ± 2.20 a
$^{15}\text{N}$ -C	0.41 ± 0.20 ab	1.97 ± 0.53 b	6.82 ± 1.96 b	9.20 ± 2.66 a
$^{15}\text{N}$ -F	0.66 ± 0.21 ab	1.80 ± 0.57 bc	8.41 ± 3.01 ab	10.87 ± 3.65 a
$^{15}\text{N}$ -W	0.19 ± 0.09 b	0.86 ± 0.38 c	2.75 ± 0.53 c	3.80 ± 0.60 b
NA-W/ $^{15}\text{N}$ -U	0.48 ± 0.13 ab	1.60 ± 0.57 bc	6.83 ± 0.82 bc	8.91 ± 1.15 ab

<sup>†</sup>  $^{15}\text{N}$  =  $^{15}\text{N}$ -enriched; NA = natural abundance; blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean; and U = urea.

<sup>‡</sup> Within columns, means ± standard deviations followed by the same letter are not significantly different (LSD;  $P < 0.05$ ).

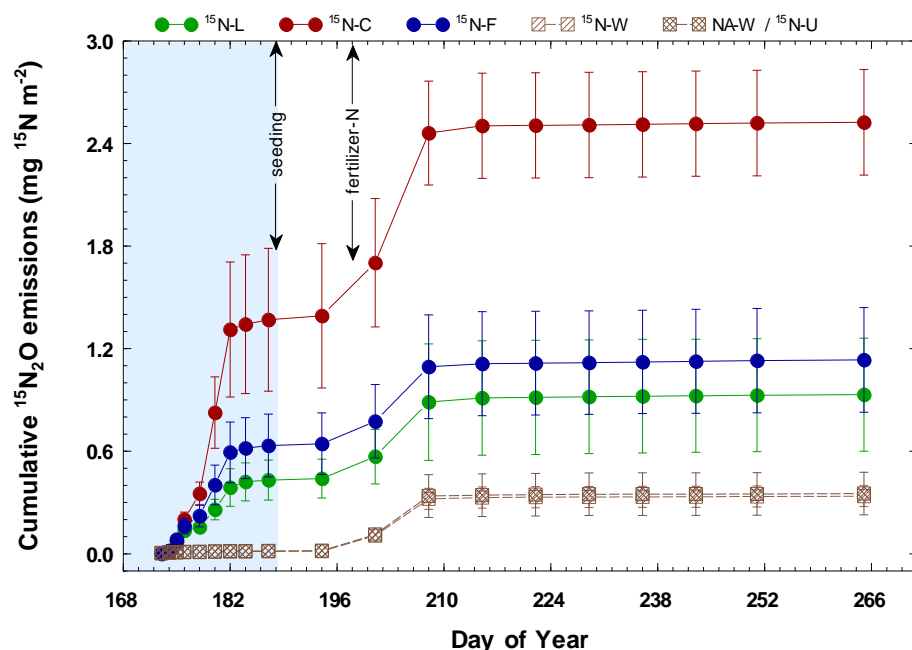
<sup>§</sup> Values reported are the weighted averages for the stems + leaves.

<sup>¶</sup> Reported as a percentage of the residue- and fertilizer-applied  $^{15}\text{N}$ .

#### 4.4.3 Residue-derived and total nitrous oxide emissions

Gas samples were collected, and analyzed for  $\text{N}_2\text{O}$  concentration (total and  $^{15}\text{N}_2\text{O}$ ) 19 times during the wheat phase of the pulse–wheat cropping sequence, with the daily  $\text{N}_2\text{O}$  flux data used to

calculate cumulative  $\text{N}_2\text{O}$  emissions (Fig. 4.5). The daily  $^{15}\text{N}_2\text{O}$  fluxes were greatest during the simulated “spring thaw” (i.e., after the residue-amended soils were removed from the freezer [DOY 173], but before seeding [DOY 188]) and after the soils were fertilized with urea (DOY 198); but were quite small once the wheat crop became established (DOY 208). Soils amended with the pulse crop residues produced significant  $^{15}\text{N}_2\text{O}$  fluxes during the thaw period, with the largest fluxes being associated with the chickpea residue. Soils amended with wheat residues, on the other hand, produced no significant  $^{15}\text{N}_2\text{O}$  fluxes during the thaw period.

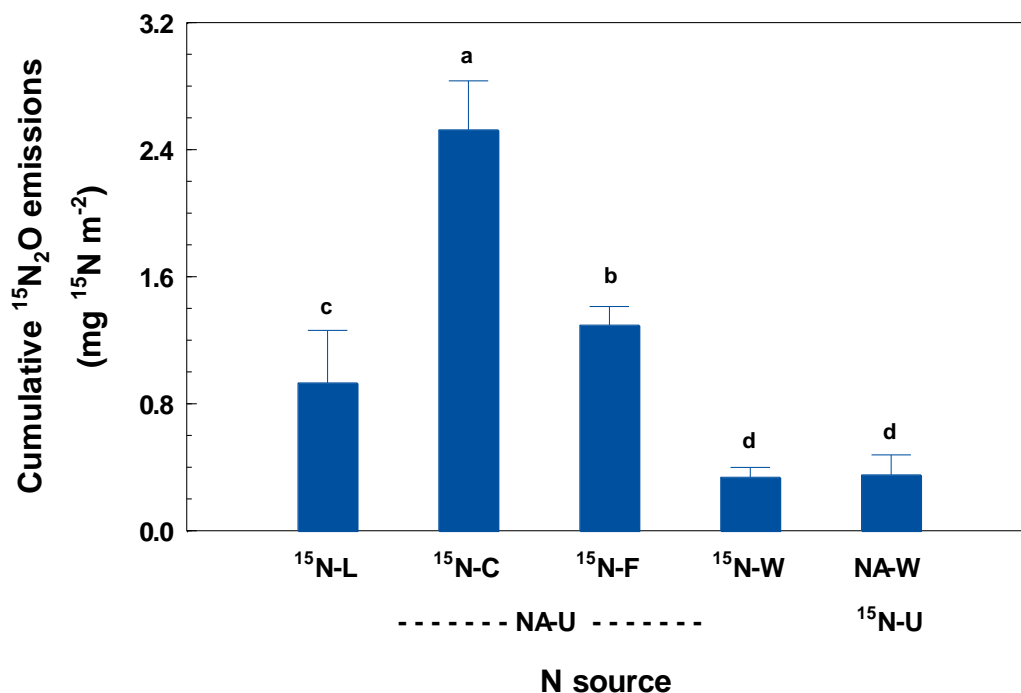


**Figure 4.5.** Cumulative residue-derived  $^{15}\text{N}_2\text{O}$  emissions from soils amended with  $^{15}\text{N}$ -labelled pulse crop and wheat residues. A soil amended with non-labelled (natural abundance) wheat residue supplemented with  $^{15}\text{N}$ -labelled urea fertilizer (equivalent to ca.  $5 \text{ kg } ^{15}\text{N ha}^{-1}$ ) was included as a reference treatment. Error bars represent one standard deviation from the mean value. The blue shaded area highlights the simulated “spring thaw” period; i.e., the time between when the pots containing residue-amended soils were removed from the freezer and they were seeded with the wheat.

Cumulative  $^{15}\text{N}_2\text{O}$  emissions from the residue-amended soils increased in the order: wheat << lentil < faba bean << chickpea (Fig. 4.6). In general, total cumulative  $^{15}\text{N}_2\text{O}$  emissions varied as a function of the amount of  $^{15}\text{N}$  added as residue (Table 4.5); i.e., there was a strong positive correlation ( $r = 0.954$ ;  $P = 0.012$ ) between the total amount of  $^{15}\text{N}$  added as crop residue and



cumulative  $^{15}\text{N}_2\text{O}$  emissions. At the same time, emission factors for the residues (i.e., the percentage of residue  $^{15}\text{N}$  added emitted as  $^{15}\text{N}_2\text{O}$ ) exhibited a weak negative correlation ( $r = -0.818$ ;  $P = 0.090$ ) with the amount of residue  $^{15}\text{N}$  added to the soils.



**Figure 4.6.** Cumulative residue-derived  $^{15}\text{N}_2\text{O}$  emissions from soils amended with  $^{15}\text{N}$ -labelled pulse crop and wheat residues. A soil amended with non-labelled (natural abundance) wheat residue supplemented with  $^{15}\text{N}$ -labeled urea fertilizer (equivalent to ca.  $5 \text{ kg } ^{15}\text{N ha}^{-1}$ ) was included as a reference treatment. Error bars represent one standard deviation from the mean value.

In addition to  $^{15}\text{N}_2\text{O}$ , total  $\text{N}_2\text{O}$  fluxes were measured during the period in which wheat was grown in the residue amended soils. In general, the emission pattern for total  $\text{N}_2\text{O}$  was the same as that observed for  $^{15}\text{N}_2\text{O}$ , with total  $\text{N}_2\text{O}$  emissions being greatest during the thaw period and after the application of N fertilizer (Appendix D, Fig. D1). Total  $\text{N}_2\text{O}$  emissions were positively correlated ( $r = 0.847$ ;  $P = 0.070$ ) with the total amount of N added to the soils (i.e., as residue and fertilizer), but there was no significant ( $P = 0.362$ ) correlation between total N added to soil and the total  $\text{N}_2\text{O}$  emission factor (see Appendix D, Table D1). Moreover, following the addition of fertilizer N on DOY 198, cumulative  $\text{N}_2\text{O}$  emissions in the soil amended with wheat or chickpea residue increased

at a much greater rate (7.74–8.71 mg m<sup>-2</sup> d<sup>-1</sup>) than it did in the soils amended with lentil (5.54 mg m<sup>-2</sup> d<sup>-1</sup>) or faba bean (4.71 mg m<sup>-2</sup> d<sup>-1</sup>) residues—suggesting a fertilizer-by-residue interaction.

**Table 4.5. Cumulative residue-derived <sup>15</sup>N<sub>2</sub>O emissions and emission factors.**

N source <sup>†</sup>	<sup>15</sup> N added as crop residue (mg <sup>15</sup> N m <sup>-2</sup> )	Cumulative <sup>15</sup> N <sub>2</sub> O emissions (mg <sup>15</sup> N m <sup>-2</sup> ) <sup>‡</sup>	EF <sub>R</sub> (%) <sup>§</sup>
<sup>15</sup> N-L	227.4	0.93 ± 0.33 c	0.37 ± 0.13 b
<sup>15</sup> N-C	696.8	2.52 ± 0.31 a	0.34 ± 0.04 b
<sup>15</sup> N-F	531.7	1.30 ± 0.12 b	0.23 ± 0.02 c
<sup>15</sup> N-W	44.1	0.34 ± 0.06 d	0.56 ± 0.10 a
NA-W/ <sup>15</sup> N-U	27.4	0.33 ± 0.13 d	0.49 ± 0.17 a

<sup>†</sup> <sup>15</sup>N = <sup>15</sup>N-enriched; NA = natural abundance; blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean; and U = urea.

<sup>‡</sup> Within columns, means ± standard deviations followed by the same letter are not significantly different (LSD; *P* < 0.05).

<sup>§</sup> Residue-derived <sup>15</sup>N<sub>2</sub>O emission factor; i.e., the percentage of <sup>15</sup>N applied as crop residue lost as <sup>15</sup>N<sub>2</sub>O = 100 × [cumulative <sup>15</sup>N<sub>2</sub>O emissions (mg N<sub>2</sub>O-<sup>15</sup>N m<sup>-2</sup>) ÷ total residue <sup>15</sup>N applied (mg <sup>15</sup>N m<sup>-2</sup>)].

## 4.5 Discussion

### 4.5.1 Seed yield of subsequently grown wheat crop

Seed yields of wheat grown in soils in which the previous crop was a pulse (i.e., lentil, chickpea, or faba bean) were higher (*P* = 0.009) than those of wheat grown in soils in which the previous crop was wheat. Indeed, on average wheat grown in soils containing pulse residues were 53% greater than those obtained when wheat was the previous crop. This is in agreement with a number of field studies that demonstrated pulse crop residues increase N availability in the soil, and thus increase the yield of a subsequent cereal crop (Beckie and Brandt, 1997; Miller et al., 2003; Felton et al., 2008; St. Luce et al., 2015). Despite these differences, the harvest index (HI) for wheat did not vary (Table 4.2) between treatments—with a mean value of about 0.49, which is on the high end of the values normally reported for field grown wheat (Unkovich et al. 2010; Dai et al. 2016). Passioura (1977) demonstrated that the HI of crops grown in a greenhouse was a function of water use—with the highest HIs occurring when there was sufficient plant available soil water to meet

the crop demand. Thus, it is likely that the relatively high HI for the wheat grown in this study reflects the fact that soil water was maintained at approximately 60% water holding capacity throughout the 13-wk growth period.

#### **4.5.2 Recovery of residue-derived nitrogen by wheat**

The recovery of residue-derived N by the wheat grown in soils in which the previous crop was a grain legume (pulse) averaged 11.2%, which is within the range of values reported by Kumar and Goh (2002) and Peoples et al. (2017); i.e., 9–14% and 12–48%, respectively. Wheat grown on wheat residue, however, resulted in a residue-N recovery of only about 4%. The percent residue-N recovery was greatest for lentil residue (13.6%) and least for chickpea residue (9.2%), though the opposite was true for the total amounts of residue-N recovered by the wheat (Table 4.4). Moreover, whereas there was no correlation between percent recovery and the amount of residue-N added ( $P = 0.549$ ), there was a strong positive correlation ( $r = 0.979$ ;  $P = 0.004$ ) between the total amount of residue-N taken up by the wheat plants and the amount of residue-N in the soils. These results suggest that the N in lentil residues is more readily available—i.e., the residues themselves are more readily mineralizable—than the N in wheat, chickpea, or faba bean residues. Indeed, Paré et al. (2000) reported that residue type strongly influenced decomposition and N mineralization, while Bremer et al. (1991) reported that lentil straw decomposed more quickly than wheat straw. Likewise, Arcand et al. (2014) reported that N mineralization was greater for the below-ground residues of lentil than for those of wheat. Data from the present study also indicate that the residues of chickpea and faba bean are more readily mineralizable than those of wheat, as was reported by Hassan et al. (2013) who found that—in a 42-d incubation study that included chickpea, faba bean, and wheat—residue decomposition increased in the order: wheat << faba bean < chickpea.

Most of the N taken up by the wheat was recovered in the grain; however, in terms of the nitrogen harvest index (NHI; i.e., the ratio of seed-N to shoot + seed-N), differences between residue treatments were not significant ( $P = 0.598$ ). At the same time, the grain N content of wheat preceded by a pulse crops was 14% to 28% greater ( $P = 0.013$ ) than that of wheat preceded by wheat. This result is in keeping with those of other studies that have shown that the grain N content of wheat produced following a pulse crop is greater than that wheat preceded by another cereal (St.Luce et al., 2016; Ross et al., 2015; Malhi et al., 2006).

In general, pulse crop residues have lower C:N ratios than wheat (Gan et al., 2011). In the present study, the C:N ratios of the pulse residues ranged from 10:1 to 14:1 for the roots and 14:1 to 18:1 for the shoots. By comparison, the wheat residues had C:N ratios of 27:1 for the roots and 69:1 for the shoots. Gupta (2016) suggested that soil mineral N is immobilized at C:N ratios greater than 20:1. Therefore, it is likely that immobilization of some of the N released during decomposition/mineralization of the wheat residues contributed to the low percent recovery of  $^{15}\text{N}$  by the wheat grown in soil amended with these residues. Pulse crops grown to maturity generally contribute less N to a subsequent crop than do pulse crops grown as green manures (Danga et al., 2013; St. Luce et al., 2015). This is because pulses grown to maturity generally have higher C:N ratios than their green manure counterparts and because most of the fixed N in the pulses is translocated to, and harvested with the seed. However, residues of mature pulses provide N benefits over a longer period of time due to their slower turnover (Beckie et al., 1997; Peoples et al., 2009; Lupway and Soon, 2015; Gan et al., 2015). Indeed, long term studies suggests crops like faba bean and lentil are likely to make consistent contributions to positive N accretion (Walley et al., 2007).

#### **4.5.3 Residue-derived nitrous oxide emissions and emission factors**

Nitrous oxide emissions associated with the soil incorporation of residues with low C:N ratios are known to be greater than those associated with the incorporation of high C:N ratio residues (Baggs et al., 2000; Huang et al., 2004; Lemke et al., 2007; Badagliacca et al., 2017). Residues with low C:N ratios are a source of N and, hence, provide inputs that drive nitrification and denitrification processes in soils. They are also known to be sources of easily decomposable C, which can act as an energy source for heterotrophic soil microorganisms. For example, low C:N crop residues have been shown to act as a substrate for nitrifiers and denitrifiers, stimulate overall microbial growth and activity in the soil, promote oxygen consumption and create temporary anaerobic microsites in the soil (Lemke et al., 2007). Thus, soil incorporation of pulse residues can result in high  $\text{N}_2\text{O}$  losses compared to non-legume residues. Based on a study with wheat residue and inorganic fertilizer, Delgado et al., (2010) suggested that crop residue with a high C:N ratio could lower  $\text{N}_2\text{O}$  emissions by immobilizing available N sources in the soil. They also found that removing high C:N crop residues increased  $\text{N}_2\text{O}$ -N losses from the soil. Indeed, in the present study, the incorporation of pulse residues into the soil resulted in  $\text{N}_2\text{O}$  emissions that were greater than those

from incorporation of wheat residues (see Figs. 4.5 & 4.6). It seems likely, therefore, that the presence of wheat residue (with a high C:N ratio) resulted in enhanced N immobilization and thus reduced the amount of available N in the soil that could have otherwise be transformed to N<sub>2</sub>O.

Nitrous oxide emission patterns are highly dynamic and are generally characterised by high degrees of spatio-temporal variability (Butterbach-Bahl et al., 2013). The peak emissions observed in this study occurred during the initial thaw period (simulating the spring thaw) and after the addition of N fertilizer. The peak events also coincided with periods of high soil moisture, which acts as a trigger for denitrification (Zhong et al., 2011). Peaks observed during the first two weeks (i.e., the thaw period) can be attributed to the formation anaerobic conditions triggered by high soil moisture content coupled with the release of readily decomposable C and mineral N from the crop residues (Dusenbury et al., 2008). This is a common scenario observed throughout the Northern Great Plains during spring snow melt (Rochette et al., 2008). The second large N<sub>2</sub>O emission peak occurred after the addition of urea-N fertilizer on DOY 198. Indeed, addition of urea fertilizer has been reported to produce an immediate increase in N<sub>2</sub>O flux (van der Weerden et al., 2016). As well, soil moisture conditions that are optimal for plant growth can facilitate N<sub>2</sub>O production via the nitrification pathway (Norton and Stark, 2011; Schimel et al., 2007). It appears, however, that this pathway was not a significant source of N<sub>2</sub>O in the present study. That is, other than during the peak events—dominated by denitrification-induced N<sub>2</sub>O production—N<sub>2</sub>O emissions were generally quite small.

Emission factors for the different crop residues (EF<sub>R</sub>) were calculated using Equation 4.1:

$$EF_R = \frac{[^{15}\text{N}_2\text{O}]}{^{15}\text{N}_R} \times 100 \quad [\text{Eqn. 4.1}]$$

where EF<sub>R</sub> is the residue-induced emission factor (%); [<sup>15</sup>N<sub>2</sub>O] is the cumulative amount of <sup>15</sup>N<sub>2</sub>O emitted (expressed as mg <sup>15</sup>N m<sup>-2</sup>) during the 13-wk growing season; and <sup>15</sup>N<sub>R</sub> is the amount of <sup>15</sup>N applied as crop residue (mg <sup>15</sup>N m<sup>-2</sup>). In general, the EF<sub>R</sub>s were within the range of values normally associated with soils in the Brown Soil Zone; i.e., between 0.2% and 0.8% (Rochette et al., 2008). However, EF<sub>R</sub>s for the pulse residues were 35% to 60% lower than the EF<sub>R</sub> for wheat—with faba bean residue yielding the lowest EF<sub>R</sub> (0.23%). Although the lentil and chickpea yielded EF<sub>R</sub>s that were quite similar (0.34% and 0.37%, respectively), total residue-derived emissions from

the chickpea residue were significantly greater than those from the lentil residue—reflecting the strong positive correlation between  $^{15}\text{N}_2\text{O}$  emissions and total residue- $^{15}\text{N}$  concentration.

In addition to residue-derived emissions, total  $\text{N}_2\text{O}$  emissions—including contributions from the crop residues, any added fertilizer-N, and the native soil N pool—also were monitored during the 13-wk growing season in the greenhouse.

In fact, a number of previous studies have suggested that the IPCC EFs should be lowered than 1% (Delgado and Follett, 2002; Malhi and Lemke, 2007; Toma and Hatano, 2007; Delgado et al., 2010; Koga, 2012; Chen et al., 2013; Shan and Yan, 2013; Delgado et al., 2010; Schwenke et al., 2015). Based on the EFs obtained in this study,  $0.38\% \pm 0.13$  and  $0.52\% \pm 0.30$  can be suggested as representative EFs for pulse crops and wheat respectively. An approximate EF value for all crop residues would be  $0.44 \pm 0.21$ . Schwenke et al., (2015) obtained comparable EFs below 1% for chickpea residue added soils (0.13-0.31%) and fertilizer added soils (0.48 -0.78%).

#### **4.6 Conclusion**

Pulse crop residues provide N credits to subsequently grown crops. The study described in this chapter evaluated the N benefits of a pulse -cereal rotation using three types of pulse crop residue that had high  $^{15}\text{N}$  enrichment (compared to natural abundance pulse residues) in order to trace the fate of fixed N in the soil and plant of the subsequently grown wheat crop. The seed yield of wheat grown on pulse crop residue (chickpea, faba bean and lentil) was 53% greater than that of the wheat grown on wheat residue. Nitrogen recovery in wheat plants was not affected by the type of N source provided and ranged from 12% to 18%. Emissions of  $\text{N}_2\text{O}$  were greater from soils amended with pulse crop residues compared to those amended with wheat residue. In general, however, the contribution of the pulse crop residues to  $\text{N}_2\text{O}$  emissions were smaller than those of N fertilizer. A large proportion of the total  $\text{N}_2\text{O}$  emissions from soils amended with pulse residues (i.e., 45% to 55%) occurred during the thaw period, compared to only about 8% to 10% of emissions from the soils amended with wheat. Emission factors varied based on crop residue type, and were generally lower for the pulse residues ( $0.38\% \pm 0.13\%$ ) than the wheat residues ( $0.52\% \pm 0.15\%$ ).

## 5. SYNTHESIS AND CONCLUSION

Pulse crops are an integral part of cropping systems in the Norther Great Plains (Walley et al., 2007). Apart from producing high protein containing grains, pulse crops provide a number of non-N benefits to both the soil and the crop grown subsequent to the pulse—promoting the sustainability of the cropping system. The overall objectives of this study were to examine the beneficial role of pulse crop residues in improving the nitrogen supply to wheat in a crop rotation and the contribution of pulse crop residues to nitrous oxide ( $\text{N}_2\text{O}$ ) emissions during the wheat year of a pulse–wheat cropping sequence. In order to do so, four pulse crops; chickpea, faba bean, lentil and pea were grown under controlled environment conditions in a rootbox system designed to provide labelled  $^{15}\text{N}_2$  gas to the soil atmosphere and produce  $^{15}\text{N}$ -enriched crop residues (Chapter 3). Above and below-ground residues of the  $^{15}\text{N}$ -enriched crops were mixed into the soil; wheat was grown during the next cropping cycle (i.e., after a freeze/thaw event), and emissions of  $\text{N}_2\text{O}$  were measured throughout the wheat growing period (Chapter 4).

The rootbox system (RB- $^{15}\text{N}_2$ ) was constructed to grow plants in a  $^{15}\text{N}_2$ -enriched soil atmosphere. The soil atmosphere was “pulse labelled” with  $^{15}\text{N}_2$ —yielding an average enrichment of 2.0823 atom%. Simultaneously, a non-fixing reference plant (wheat) was grown in soil amended with  $^{15}\text{N}$ -labelled urea fertilizer, and maintained under controlled environment conditions. Three week old seedlings were transplanted to the labelling system and harvested after 10 weeks. Above and below ground plant components of all plants had significant  $^{15}\text{N}$  enrichment levels (i.e., ranging from about 0.76 to 1.89 atom%  $^{15}\text{N}$ ) and all plants except pea produced sufficient amounts of dry matter to be used as crop residue for the next crop in the sequence (i.e., wheat). Due to poor nodulation and retarded vegetative growth, pea plants were not successfully developed in the RB- $^{15}\text{N}_2$  system. Therefore, pea residue was not used in the wheat phase of the study. Biological N fixation by the pulse crops was determined from the  $^{15}\text{N}$  enrichment in the above-ground (stems + leaves + pods) and below-ground (roots) components of the harvested plants. The percentage of N derived from the atmosphere (%Ndfa) in above-ground components increased in the order: lentil < faba bean  $\approx$

chickpea; while in the below-ground components, the %Ndfa increased in the order: lentil < faba bean < chickpea.

For chickpea, lentil and faba bean the partitioning of  $^{15}\text{N}$  in the above-ground components was relatively constant. Conversely, in the below-ground components,  $^{15}\text{N}$  enrichment was greater for chickpea than for either lentil or faba bean. Below-ground biomass production by lentil was about one-third that for chickpea and faba bean; thus even though  $^{15}\text{N}$  enrichment in the below-ground component of the lentil and faba bean were similar, the total amount of fixed N ( $\text{mg N pot}^{-1}$ ) available to the subsequent crop was significantly lower for lentil than for either faba bean or chickpea. However, the enrichment levels in the soil were significantly lower than those in the plant components. Hence, it can be assumed that most of the fixed N was in the plant residues, and thus transferred to the next phase of the study.

Above-ground and below-ground residues obtained during the pulse phase of the pulse-wheat cropping sequence were mixed with soil (e.g., chickpea soil was mixed with roots and straw, extracted from harvested chickpea plants) and frozen at  $-20^{\circ}\text{C}$  for two weeks. In order to simulate spring thaw conditions, the soils were brought to room temperature, and maintained at these temperatures, for two weeks before being seeded with wheat. Three pulse residue treatments (chickpea, faba bean and lentil) and two wheat treatments ( $^{15}\text{N}$ -enriched wheat residue and non-labelled [natural abundance] residue amended with  $^{15}\text{N}$ -labelled urea) were used as soil amendments to grow wheat. Wheat was then direct seeded into the soil, and the plants were harvested after 13 weeks. Seed yields of wheat were greatest when the wheat was preceded by a pulse crop compared to when the previous crop was wheat. Likewise, recovery of residue-derived N was 2.5 to 3.5-times greater for wheat on pulse residue than for wheat on wheat residue.

Nitrous oxide emissions from the soils amended with pulse residue were greater than those from the soil amended with wheat residue. Indeed, both total and residue-derived  $\text{N}_2\text{O}$  emissions observed under chickpea were greater than those from soils amended with faba bean or lentil residues. Due to their low C:N ratios, the pulse crop residues likely decomposed faster than the wheat residues and, thus, provided more readily available N that subsequently underwent nitrification and/or denitrification. Residues with high C:N ratios, such as wheat, promote organic matter accumulation and C sequestration and emit less  $\text{N}_2\text{O}$  compared to the N in synthetic fertilizers. However, the type of soil amended was not the governing factor for  $\text{N}_2\text{O}$  emissions



observed in the wheat growing soils. Anaerobic conditions occurred in the soil during the spring thaw period and the incorporation of inorganic fertilizer created the highest peaks in N<sub>2</sub>O emission fluxes consistently under all treatments. The emission factors (EFs) derived for all treatments were lower than the IPCC default EF (1.00%), and were within the range of values used in the Tier II methodology used to calculate the Canadian national greenhouse inventory. Moreover, EFs were generally lower for the pulse residues than for wheat—increasing in the order: faba bean < lentil ≈ chickpea < wheat.

## **5.1 Future research**

The soil atmosphere labelling method could be modified to perform more intensive experiments on N fixation using a higher number of plants over a longer period of time. Information obtained from this study shows N fixation and distribution patterns at only a single time point (i.e., at harvest). By destructively sampling plants at different growth stages additional insights into the dynamics of Ndfa% and percentage of N derived from the roots (Ndfr%) can be obtained. Indeed, the RB-<sup>15</sup>N<sub>2</sub> rootbox system allows for near-quantitative recovery of the plant roots, and thus produces more accurate estimates of the effect of root exudation and Ndfr on the soil N pools.

By the end of the cereal phase, partially decomposed crop residues were observed in the soils. Analysis of this material for their <sup>15</sup>N, total N and total C content could have produced information on the mineralization status of the residues. After harvesting the second crop (wheat) the same soil could be used to grow a different crop type such as an oil seed crop to evaluate long term N benefits of pulse crop residues. It can also be tested for different fertilizer rates in order to test for the potential of residues to replace mineral fertilizer. As N mineralization is closely associated with C content in the plant <sup>13</sup>C could also be tracked using this system. By this way, we can label the pulse crops with both C and N isotopes to track the residue decomposition and mineralization / immobilization process in the soil. It would also allow to analyse CO<sub>2</sub> emissions in addition to N<sub>2</sub>O which can provide more information on GHG emissions occur in pulse residue amended soils.

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## APPENDIX

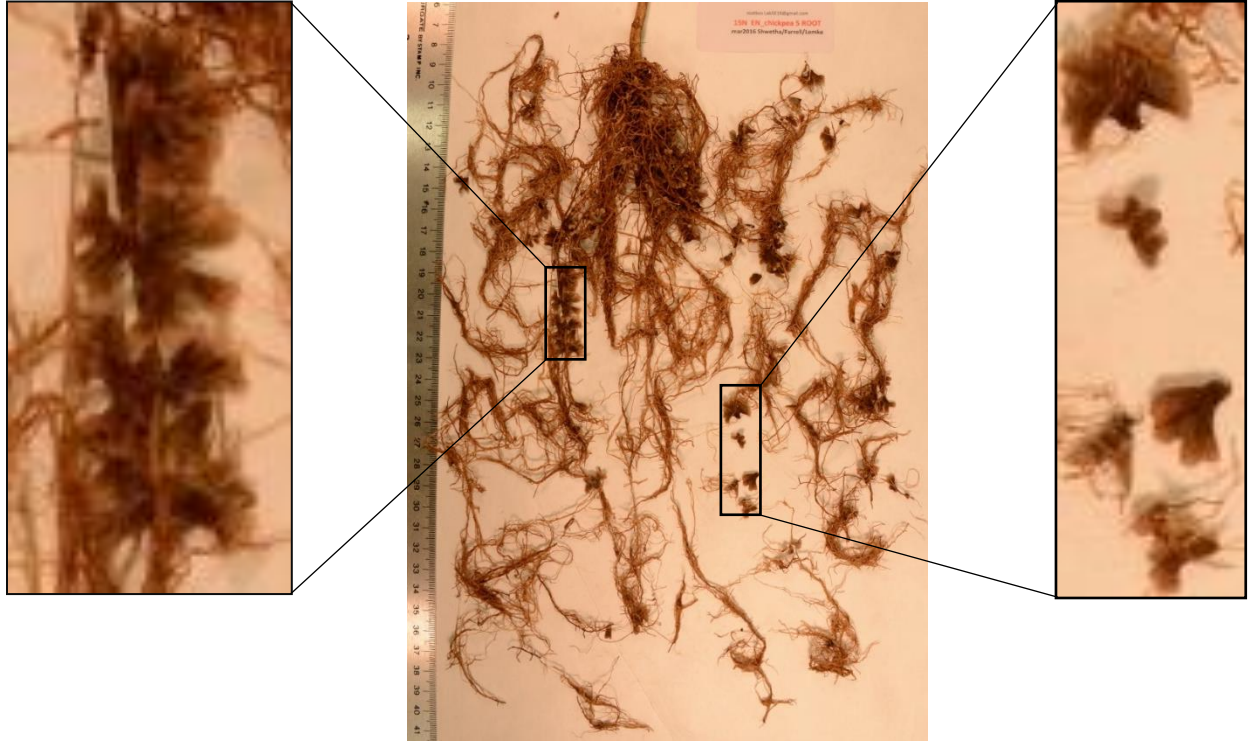
### Appendix A: RB- $^{15}\text{N}_2$ System Designed to Execute $^{15}\text{N}$ -Labelling of Pulse Crops



Figure A1. The fully assembled RB- $^{15}\text{N}_2$  system showing the expansion bladders (A), light bank (B), water inlets (C), moisture sensors (D) and gas inlets (E).

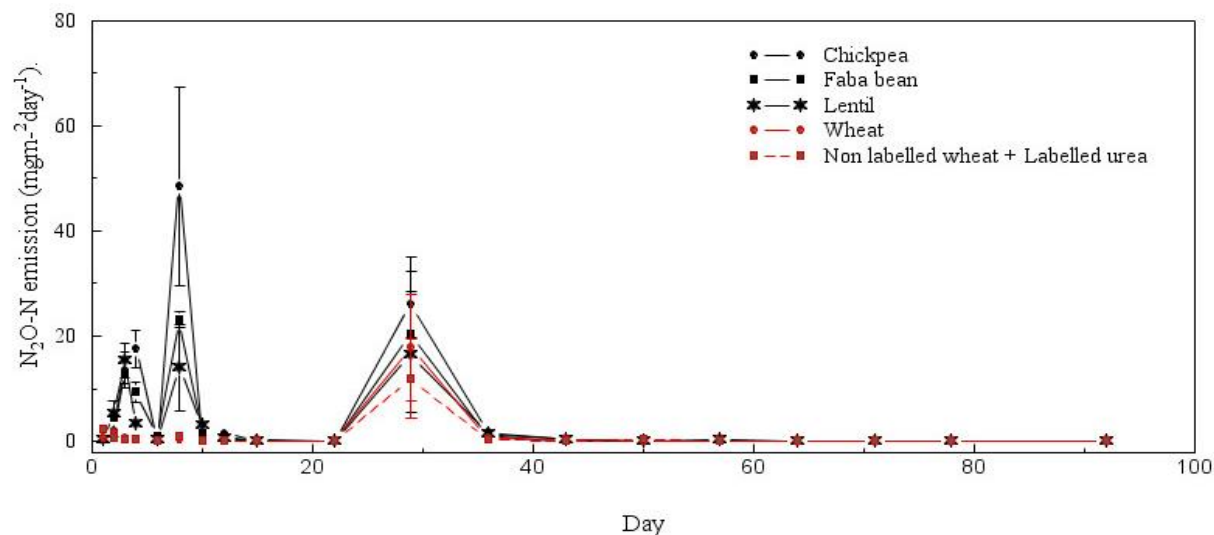


## Appendix B: Roots of pulse crops grown in the RB-<sup>15</sup>N<sub>2</sub> Rootbox System

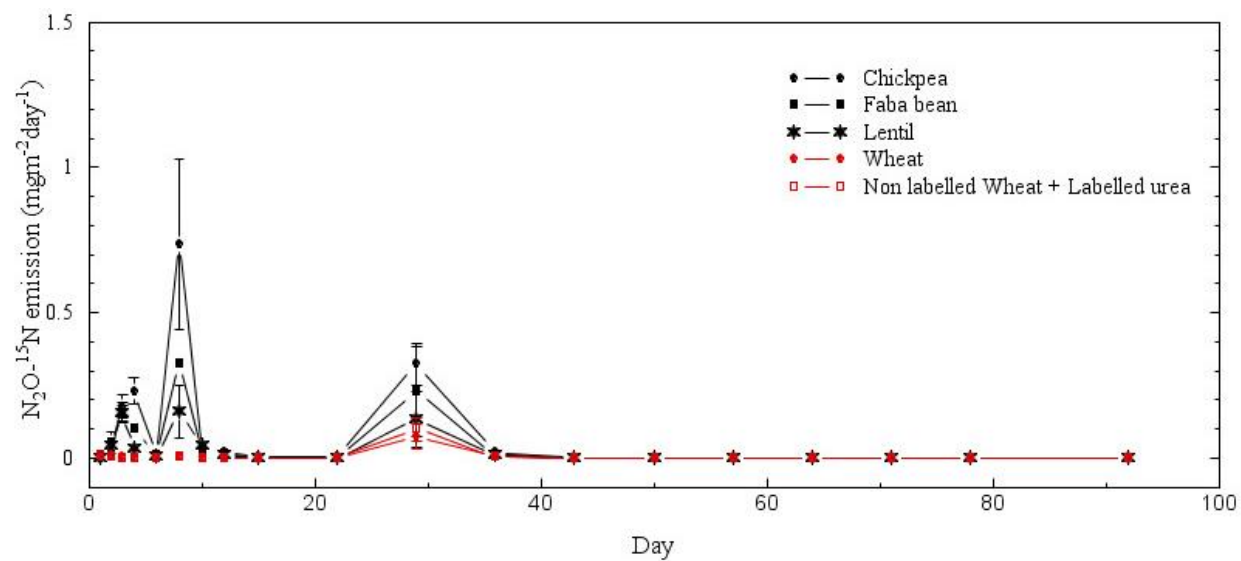


**Fig. B1.** Root system showing clusters of root nodules (A and B) observed in harvested chickpea plants grown in the RB-<sup>15</sup>N<sub>2</sub> system.

## Appendix C: Nitrous Oxide Emission Patterns for Total and Residue-derived N

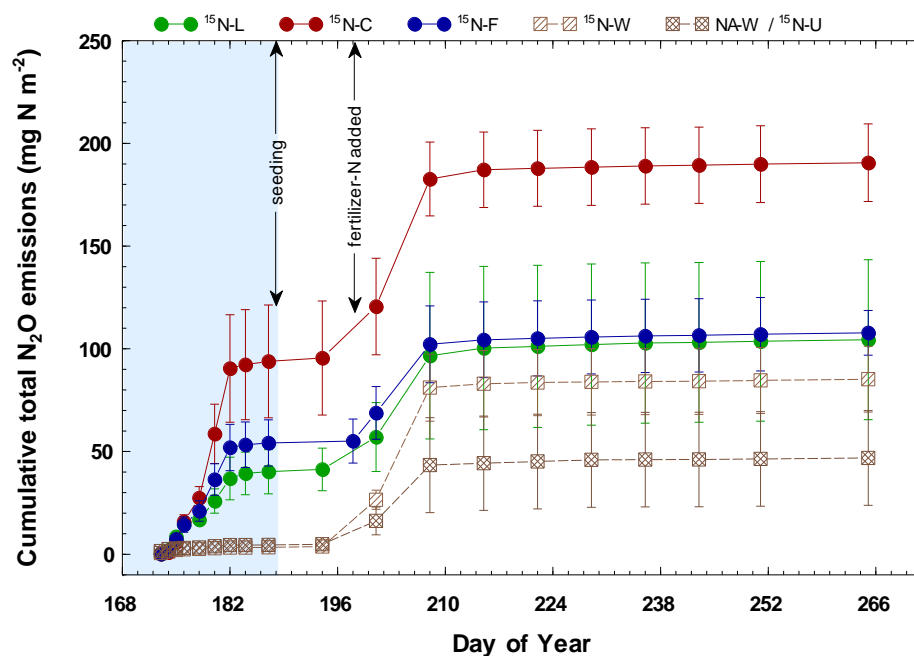


**Fig. C1.** N<sub>2</sub>O-N emissions (mg m<sup>-2</sup> day<sup>-1</sup>) from soil amended with <sup>15</sup>N-labelled residues of chickpea, faba bean, lentil and wheat and soil amended with non-labelled (natural abundance) wheat residue plus <sup>15</sup>N-labelled urea fertilizer (equivalent to *ca.* 5 kg <sup>15</sup>N ha<sup>-1</sup>). Emissions from soils amended with pulse residues are shown in black; emission from soils amended with wheat residues are shown in red.



**Fig. C2.**  $\text{N}_2\text{O}$ - $^{15}\text{N}$  emissions ( $\text{mgm}^{-2}\text{day}^{-1}$ ) from soil amended with  $^{15}\text{N}$ -labelled residues of chickpea, faba bean, lentil and wheat and soil amended with non-labelled (natural abundance) wheat residue plus  $^{15}\text{N}$ -labelled urea fertilizer (equivalent to *ca.*  $5 \text{ kg } ^{15}\text{N ha}^{-1}$ ). Emissions from soils amended with pulse residues are shown in black; emission from soils amended with wheat residues are shown in red.

**Appendix D: Cumulative total N<sub>2</sub>O emissions from soils amended with <sup>15</sup>N-labelled pulse crop and wheat residues.**



**Figure D1.** Cumulative total N<sub>2</sub>O emissions from soils amended with <sup>15</sup>N-labelled pulse crop and wheat residues. A soil amended with non-labelled (natural abundance) wheat residue supplemented with <sup>15</sup>N-labeled urea fertilizer (equivalent to *ca.* 5 kg <sup>15</sup>N ha<sup>-1</sup>) was included as a reference treatment. Error bars represent one standard deviation from the mean value. The blue shaded area highlights the simulated “spring thaw” period; i.e., the time between when the pots containing residue-amended soils were removed from the freezer and they were seeded with the wheat.

**Table D1. Cumulative total N<sub>2</sub>O loss and the emission factors calculated for the losses.**

<b>N source<sup>†</sup></b>	<b>Total N<sub>2</sub>O emissions (mg N m<sup>-2</sup>)<sup>‡</sup></b>	<b>EF (%)<sup>§</sup></b>
<sup>15</sup> N-L	104 ± 39 b	0.55 ± 0.21 b
<sup>15</sup> N-C	191 ± 19 a	0.46 ± 0.05 bc
<sup>15</sup> N-F	108 ± 11 b	0.28 ± 0.03 c
<sup>15</sup> N-W	85 ± 16 b	0.80 ± 0.15 a
NA-W/ <sup>15</sup> N-U	47 ± 23 c	0.40 ± 0.20 bc

<sup>†</sup> <sup>15</sup>N = <sup>15</sup>N-enriched; NA = natural abundance; blank = non-planted soil without residue or fertilizer; W = wheat; L = lentil; C = chickpea; F = faba bean; and U = urea.

<sup>‡</sup> Within columns, means ± standard deviations followed by the same letter are not significantly different (LSD; *P* < 0.05).

<sup>§</sup> Nitrous oxide emission factor; i.e., the percentage of applied N lost as N<sub>2</sub>O = 100\*[cumulative N<sub>2</sub>O emissions (mg N<sub>2</sub>O-N m<sup>-2</sup>) ÷ total N applied (mg N m<sup>-2</sup>)].